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ADVANCES IN PEST CONTROL RESEARCH

Volume II

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ADVANCES IN PEST CONTROL RESEARCH

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VOLUME II

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ONULP

Introduction to the Series

Pests—organisms that compete with man for his food supply, damage his possessions, and attack his person—are of vital concern to all mankind. Included in this category are some thousands of economically destructive species of plants and animals, variously classified as viruses, bacteria, fungi, weeds, protozoa, nematodes and other helminths, molluscs, insects and other arthropods, fish, birds, and rodents. The accumulated attacks of all of these pest organisms cost the human race incalculable damage in direct loss of vital foodstuffs, in enormous expenditures for pest control activities, and in pestilence and disability.

Pest control in its broadest sense is a continuous warfare against man's competitors, carried out on countless fronts with operations ranging in scope from those of the housewife and home gardener to the global efforts of the Food and Agriculture Organization and the World Health Organization of the United Nations.

Throughout the centuries, pests have demonstrated amazing plasticity in their abilities to circumvent man's best efforts to control them, and presently it is abundantly clear that research in pest control not only is essential to ensure human enjoyment of the fruits of the earth but also is capable of yielding unsuspected dividends in terms of an expanding population with abundance for all.

Pest control research currently occupies the attention of a significant portion of the world's scientific manpower, at work in university, governmental, and industrial laboratories, and in field experimentation the world over; increasing the basic knowledge of our animal and plant competitors and developing and applying more suitable and effective measures for their control. Although these research endeavors are often carried out under the various classical disciplines of plant pathology, entomology, economic botany and zoology, it is apparent that they have much in common. This has been recognized by the employment of such terms as plant protection or Pflanzenschutz research. Furthermore, progress in pest control research is directly dependent upon the ancillary disciplines of chemistry, physics, ecology, and pharmacology. It is therefore

33764

to this broad field of endeavor that *Advances in Pest Control Research* is addressed.

The subject matter will be selected from recent significant research trends related to all phases of pest control, with emphasis on the fundamental aspects. As in other "Advances," the writers will be specialists in the various fields who will be urged to contribute not only comprehensive reviews but also critical evaluations of new concepts and developments. It is hoped that by the encouragement of the presentation of personal viewpoints, *Advances in Pest Control Research* will become a stimulating vehicle for the fostering of new lines of research as well as a continuing reference work for the research worker, the teacher, and the student. Advice and counsel leading to the best approaches to these objectives are earnestly solicited.

R. L. METCALF

Riverside, California
June, 1957

CONTENTS

The Fluid Kinetics of Application of Pesticidal Chemicals
By R. P. Fraser 1

Innate Toxicity of Fungicides
By S. E. A. McCallan and Lawrence P. Miller 107

Research Advances in Seed and Soil Treatment with Systematic and Nonsystematic Insecticides
By H. T. Reynolds 135

Isotope Dilution Techniques for the Determination of Pesticide Residues
By Carl T. Redemann and Richard W. Meikle 183

Wool Digestion and Mothproofing
By D. F. Waterhouse 207

The Relation of Chemical Structure to Activity for the 2,4-D–Type Herbicide and Plant Growth Regulator
By R. L. Wain 263

Chemical Structure and Activity of DDT Analogues with Special Consideration of Their Spatial Structures
By Randolph Riemschneider 307

The Spread of Insecticide Resistance in Pest Species
By A. W. A. Brown 351

Subject Index 415

Cumulative Index of Volumes I–II 425

THE FLUID KINETICS OF APPLICATION OF PESTICIDAL CHEMICALS*

By R. P. FRASER, *London, England*

CONTENTS

I. Liquid Atomization 2
  A. Spraying in Agriculture 4
  B. Spray Requirements for Applying Crop Protection Chemicals 4
II. Methods of Atomization 9
  A. Classification of Atomizers 10
  B. The Mechanics of Producing a Liquid Sheet 12
    1. Production of the Sheet by Pressure 12
    2. Production of the Sheet by Centrifugal Force (Rotary Atomizers) 28
    3. Production of the Sheet by Air Blast (Twin-Fluid Atomizers) 32
III. Principles of Disintegration 38
  A. Photographic Methods of Research 38
  B. Mechanism of Disintegration 42
  C. Effect of Liquid Properties on Disintegration 54
  D. Effect of Additives on Disintegration 54
    1. Effect of Suspensions 55
    2. Effect of Wetting Agents 57
    3. Effect of Visco-elasticity 58
IV. Projection and Disintegration of Drops in a Gaseous Stream 61
  A. Gaseous Flow in Nozzles 63
    1. Power Required for Moving Air 68
  B. Disintegration of Drops Traveling in a Gaseous Medium 68

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* This review is aimed to inform the agricultural engineer of the results and trends of modern fundamental research in the fluid mechanics of the dissemination of insecticides in liquid form and to provide him with design data which he may employ without further reference to the literature. It should also be of interest to the student who enters the field without knowledge of the now considerable literature on the subject of atomization. An attempt has been made not only to bring him up to date regarding research into these problems but also to indicate where new knowledge is required. Inevitably, the author has introduced his own views at various points, for which he offers no apology.

V. Drop Size in Sprays 73
  A. Drop Size Determination Methods 73
  B. Rate of Increase of Liquid Surface by Atomization 76
  C. Drop Size from Different Atomizer Designs 76
    1. Pressure Atomizers 78
    2. Rotary Atomizers 85
    3. Twin-Fluid Atomizers 88
VI. Spatial Dispersion and Deposition of a Spray 96
References 103

Man, by the instrument of his greater mobility, has become his own greatest enemy in the dissemination of diseases and pests to new territory; thus, the study of the preparation and the kinetics of projection of liquid chemical sprays and dusts onto crops and buildings is of ever-increasing importance. To obtain control of methods and economy in distribution of active chemicals requires a close study of the mechanics of fluid flow and disintegration. Control of particle size and dispersion, improvements in projection and in control of the ambient conditions in the region of the nozzle, combined with parallel work of biological and entomological control, will undoubtedly yield better methods of application and greater economy in the future.

I. Liquid Atomization

The disintegration of liquids by the process of atomization is commonly employed in three major industries: combustion engineering, chemical engineering, and agriculture. The atomization of liquid fuels for combustion is about a century old, and interest is confined to hydrocarbons having a small variance of physical properties. In agriculture the interest is mainly in aqueous solutions and means to convey the spray a long distance, whereas in chemical engineering a knowledge is required of the effect of a wide variation of physical properties of the liquid and atmosphere. The chemical engineer has to apply his knowledge of the handling of a multitude of liquids, ranging from chemical solutions, emulsions, and dispersions to slurries and even gels. The majority of atomizers commonly employed have been designed for simple liquids, namely, inviscid Newtonian liquids. When they are employed for slurries, pastes, and liquids having anomalous or non-Newtonian properties, there is a great change; sometimes there is also deterioration in performance, and, in many cases, the atomizers may be rapidly eroded and damaged to the point of uselessness.

To understand the processes of disintegration the engineer must study the fluid kinetics of rapidly moving liquid jets and sheets, in order to design suitable equipment. Much is to be gained by the agricultural engineer from a study of the various types and designs of atomizers so that a suitable selection can be made for the duty to be performed.

The general purpose of spraying is to increase the surface area of a liquid mass in order to hasten some physical or chemical process. In agriculture the process is either one of dispersing the volume into a much larger volume of air or one of dispersing the volume over an area so that the increased liquid surface is transferred to another surface. While the production of a large liquid surface fulfills an important requirement of the process, efficient operation can only be achieved if the drops are of a specified size and, what is perhaps more important, have a high degree of uniformity. Only if these conditions are met can the design be on a rotational basis.

The large number of dimensional variables in the atomizer, the variation in the liquid properties and in the hydraulics of flow, and also the changes in the ambient surroundings of the spray are responsible for the complexity of the phenomena associated with spraying. Although considerable research effort has been directed toward the assessment of the effects of the many variables, the difficulties encountered both theoretically and experimentally limit the scope of investigation. Much of the research on sprays is concerned with the collection of empirical performance data, and the results of various workers and their interpretations of experimental evidence conflict in many instances. This is not surprising when it is realized that there are few, if any, generally accepted experimental procedures.

The flow inside and outside the nozzle can be analyzed and related to the design, and a theory of flow can be developed. The pattern and uniformity of sprays can be determined. The spray angle can be measured, and the manner of liquid disintegration can be studied by photographing the liquid sheet. Part or all of the spray can be caught and a direct or indirect size analysis made. The uniformity of spray deposition can be analyzed by spraying liquid from a stationary or moving nozzle onto a horizontal or vertical surface and the relative deposition volumes and transit losses obtained.

A. SPRAYING IN AGRICULTURE

In agriculture it has been usual to distribute the active chemical by spraying dilute solutions through nozzles at high flow rates (i.e., up to 1200 gal./hr. per nozzle) in conditions which may vary widely. The type of crop, the layout of crop, the atmospheric conditions, and the form of the mechanical equipment supplying the energy for distribution may all vary from time to time. This variability of field conditions and the need, in many cases, to ensure complete coverage of the herbage make it necessary to use very much more solution than should theoretically be required. Spraying to "runoff" usually wastes 95% of the liquid and often does not completely wet the plant. The great difference in trajectory and distance of throw required by ground and tree crops adds further variation.

The need to reduce the quantities of water used in spraying in arid areas brought about new requirements. The difficulties of transporting large quantities of water to the area to be sprayed, on the one hand, and the fact that only a few drops of plant regulator hormone are required per leaf or plant, on the other, emphasized the need for nozzles capable of distributing very small quantities of liquid uniformly. Thus it became necessary to atomize and distribute, in small drops and with great uniformity, very small volumes of 0.1–2 gal./hr., over large areas of ground—for example, 1–10 gal./acre.

Low-volume spraying requires that the drops be small because the area covered by a given volume of liquid increases as the drop size gets smaller. Small drops mean drift and low dynamic catch when spraying crops. With pressure nozzles, small drops mean small orifice sizes and high accuracy in production. Thus atomizers using gaseous energy are attractive because the liquid orifice can be large.

B. SPRAY REQUIREMENTS FOR APPLYING CROP PROTECTION CHEMICALS

Some crop protection chemicals require maximum deposition; others, for space sprays, require the maximum time in suspension. Spraying in agriculture and horticulture, therefore, falls under a number of heads—each with different and characteristic atomizer requirements—such as in crop sprayers, orchard sprayers, aircraft sprayers, spray and mist blowers, and aerosol generators for space spraying. In each application a number of nozzle sizes and designs are required to obtain the desired effect. There is no universal nozzle or atomizer for all purposes, although some attempt has been made to design reliable and sturdy pressure nozzles with interchangeable parts to enable the user to satisfy a number of spraying requirements.

Ripper (85) enumerates the types of spray required for different operations in crop protection and defines them as (*a*) a cover of fine drops deposited on plant and soil from space sprays, systemic sprays, and contact sprays; and (*b*) the deposit of a number of comparatively large drops on vegetation. Space sprays require a drop size of 1–30 μ; systemic and fungicidal sprays without air assistance require drops greater than 100 μ; and contact sprays, such as weed-killers, 100–300 μ. With air assistance drops can be reduced to a mass median diameter (MMD) of 60 μ (say, 47 μ surface mean diameter (SMD)). Field experience appears to show that the optimum droplet size for good coverage by ground sprays without air assistance is 80–150 μ MMD. Spraying from aircraft requires a larger drop, namely, 100–200 μ MMD without air assistance.

Two of the factors which may affect the availability of an insecticide to an insect are the drop size and drop size dispersion. So far little is known about the effect of drop dispersion either during flight or on a surface. Latta *et al.* (58) investigated conditions in flight and expressed the size dispersion requirements in terms of drop size. They showed, in wind tunnel experiments, that if

$$d^2V = 2000 \tag{1}$$

where d is in microns and V is in miles per hour, the maximum deposition is obtained on the insect, and that for normal wind speeds drops of 10–20 μ are required.

For space sprays, drops have to be small enough to remain airborne, but large enough to impact the insect during flight and not be carried round it by the air currents. Similar requirements obtain for outdoor spraying where the insect may be protected by a forest canopy. For this type of application drops above 100 μ are considered useless.

With oil emulsions it is difficult to generalize about the effect of drop size on toxicity. It appears that the oil content and the physical state of the emulsion may be more important than the drop size. Griffin *et al.* (38) showed that a 0.5% gas oil emulsion sprayed on green fly was more toxic when it contained drops of 8–12 μ than when the size was 2 μ or less. Further, oil emulsions used as cattle sprays have shown highest toxicity when the spray contained the greatest number of large drops (89). On the other hand, Beran (3), using emulsions in field trials on fruit trees, showed that the highest toxicity is reached with the minimum drop size. These various effects may be due to the interaction of drop size with insecticide property combined with the variable losses of small drops in transit. Hadaway and Barlow (39) have studied the relation of actual drop sizes on surfaces to toxicity using contact insecticides (wettable powder DDT, methoxychlor, etc.). They have shown that reducing the drop size down to 10 μ increases the toxicity, an optimum effect occurring with drops between 10 and 20 μ, but there is also a variation effect due to the type of surface; 1–10 μ is not effective on plaster board but is effective on mud block. Highly toxic compounds are less influenced by drop size. It is clear that although something is known of the effect of the size of residual particle on toxicity, little is known of the desirable liquid drop size which should act as the carrier.

The increasing use of living insecticides will no doubt bring about new requirements in spraying, and in some cases it may produce a solution of the vexing problem of aerial drift because such living insecticides only attack the insect or insects to be destroyed. In some cases it has been found that nematodes carrying bacteria deadly to insect pests can be sprayed at high pressure through small orifices without changing their biological effectiveness. It is quite certain that the greater efficiency of biological control over chemical control may enable much larger areas to be successfully covered with much less effort and equipment. Furthermore, in searching out its prey, the bacterium can convert a relatively inefficient mechanical distribution, through spraying, into a highly efficient and lethal method of control. Because the bacterium is self-spreading, the drop size should not need to be as small as is required with chemical solutions. It should also be possible to spray a medium which both has the physical properties of a gel—or at least high viscosity—and is a suitable culture or carrying medium for the bacterium. When successful, the method can be cheaper than chemicals since the first cost is the last.

The type of duty an atomizer has to perform can be classified according to the volume of flow per unit time at a particular pressure and whether the spray has to project over a short or long distance. Atomizer nozzle requirements for crop protection purposes can thus

TABLE I
Atomizer Requirements for Crop Protection

Nozzle flow rate, gal./hr.	Pres., p.s.i.	FN	Drop size (SMD), μ			Type of atomizer (See Table II)	Character of use
			Short-range spraying, under 6 ft.				
1. Very small							
0.1–1.0			1–30; air atomized $1^1/_2$–5 lb. air/lb. liquid; air velocity 300–1000 ft./sec.			Medium-pressure twin-fluid or blast atomizers	Aerosol space sprays
2. Small							
			Fan ($\theta = 90°$)	Swirl ($\theta = 90°$)			
2.0	25	0.4	147	110		Small swirl spray nozzles	Fungicide and systemic sprays
	50	0.28	104	78			
	100	0.2	74	55			
4.0	25	0.8	186	139		Small single hole nozzles	Ground sprays
	50	0.57	132	99			
	100	0.4	93	70			
8.0	25	1.6	234	175		Medium-pressure twin-fluid; 0.1–0.3 lb air/lb. liquid	>100 μ without air assistance
	50	1.13	165	123			
	100	0.8	117	88			
16.0	25	3.2	295	220			
	50	2.27	210	156			
	100	1.6	147	110			
3. Medium							
			Impact (Jet = 30°)	Swirl ($\theta = 90°$)			
15	60	1.94	370	139		Swirl spray nozzles	Large insecticidal sprays
	120	1.36	260	98			
	240	0.97	(185)	70			
30	60	3.9	465	175			
	120	2.7	327	123			
	240	1.94	(240)*	90			
60	60	7.75	(585)	(220)		Impact nozzles	Contact weed killers; 100–300 μ
	120	5.4	410	155			
	240	3.9	(290)*	112			
120	60	15.5	(740)	(280)	215		
	120	10.8	(520)*	(195)	180		
	240	3.9	(370)*	(140)	(130)		

(*continued*)

TABLE I (*continued*)

Nozzle flow rate, gal./hr.	Pres., p.s.i.	FN	Drop size (SMD), μ		Type of atomizer (See Table II)	Character of use
240	60	31.0	(930)*	290		
	120	21.6	(655)*	225		
	240	15.5	(465)*	(185)		
			Long-range spraying, over 6 ft.			
4. Very small to medium						
0.1–15			40; air- or pressure-atomized and air-assisted		Swirl spray nozzles Spinning disks Low pressure twin-fluid	Mist blowers
5. Large						
			Impact (Jet = 30°)	Swirl (θ = 90°)		
300	200	21.3	(550)*	(207)	Swirl spray nozzles	Orchard and tree spraying
	400	15.0	(390)*	(160)*		
	600	12.2	(315)*	(140)*		
600	200	42.6	(695)*	(280)*	Impact nozzles	Spraying to "runoff"
	400	30.0	(485)*	(220)*		
	600	24.4	(400)*	(190)*		
1200	200	85.2	(875)*	(375)*		
	400	60.0	(620)*	(290)*		
	600	48.8	(500)*	(250)*		
6. Very large						
			Swirl (θ = 90°)	Simple jets		
3000	30	550	1100	1820*	Simple jets	Overhead irrigation
	90	315	740*	1050*	Swirl spray	
	180	225	(575)*	740*	Impact nozzles	
6000	30	1100	(1470)	1820*		
	90	630	990*	1050*		
	180	450	(780)*	745*		

be classified into short and long range; small, medium, and large flow rate; high and low pressure.

Table I gives the characteristic drop size from various atomizers at different flow rates and pressures. The figures are calculated for well-designed nozzles using water of viscosity 1 centistoke and surface tension 73 dynes/cm. The surface mean diameter (SMD) has been chosen to express the spray character because this mean, which expresses a surface property, is more widespread in engineering practice than the mass median diameter (MMD), which has so far been more commonly used in agricultural literature (see Section V.A.). The drop sizes in the table have been calculated from the various formulas for water given in Section V. The figures in parentheses are extrapolations (in many cases extreme extrapolations) from the known data and illustrate only too well the present lack of precise knowledge. With simple jets and impact nozzles, where the projected velocity of the drop may be nearly that of the theoretical velocity corresponding to the pressure used, the large drop diameters given in parentheses followed by an asterisk are in almost all cases above the critical size for that velocity; thus they will be further shattered to smaller drops by air fraction (see Section IV.B).

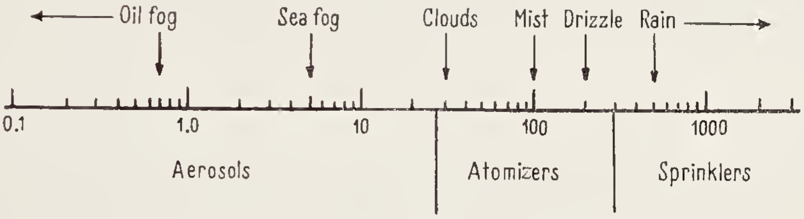


Fig. 1. Drop size spectrum, in microns.

It will be seen from Table I that crop protection requires the use of drop sizes corresponding to the entire natural drop spectrum, and the different kinds of spray have derived their names from their drop size equivalent in nature, as given in Fig. 1. With drops in free fall, as in rain, the maximum possible size is 8–9 mm. A suddenly applied air blast at sonic velocity realizes a survival diameter of 5–6 μ, and this size is the practical minimum average size for drops produced in ordinary spraying devices.

II. Methods of Atomization

A liquid projection nozzle, as may be used in a fire appliance for projecting a jet of water, or a nozzle employed in a liquid fuel projector for flame throwing, is not an atomizer, although the liquid jet must eventually break up, mainly because of air friction. Such nozzles may be arranged with turbulence rectifiers before the orifice and are specifically designed to achieve an undisturbed jet to enable it to be projected to the maximum distance, as may be required for overhead irrigation. An atomizer, on the other hand, is precisely designed to cause the liquid to break up as quickly and as uniformly as possible. An atomizer may not necessarily cause the liquid to break up into the smallest drops, but a necessary feature is that it should disintegrate the liquid in a controlled manner.

The engineering function of an atomizer is therefore twofold: first, to cause the liquid to accelerate and make it issue as a sheet and then disintegrate and, second, to direct the resulting particles in a controlled direction. According to this definition, an atomizer is a device which not only disintegrates a liquid into a spray of drops, but also controls the spatial distribution of the spray.

A. CLASSIFICATION OF ATOMIZERS

The most convenient classification of atomizers is according to the source of energy employed, and they divide naturally into three basic types: those using (*1*) pressure energy, (*2*) centrifugal energy, or (*3*) gaseous energy. But it must be noted that there is no fundamental difference in the mechanism of breakup of the liquid among the different types.

In the present literature on atomization considerable confusion exists as regard terminology, and it would be a great help in the future if a standard terminology could be established. Even the main classification headings are not agreed upon, pressure atomization being very often referred to as "hydraulic" or "mechanical" atomization, and gaseous atomization as "air" or "pneumatic" atomization. The use of the term "hydraulic" is unfortunate, for all atomizers deal with liquids and are, therefore, hydraulic instruments. The term "mechanical atomizer" is commonly employed in America but is easily confused with spinning disks and cups driven by mechanical means. In Table II spinning disks or cups are referred to as rotary atomizers, not as centrifugal atomizers, which term has been commonly applied in the United States to the swirl spray nozzle. The term "air or steam atomizer" is not used because any gas can be employed, and the term "twin-fluid or blast atomizer" is preferred. The number of terms used in the minor classifications of pressure

TABLE II
Classification of Atomizers

Pressure energy used for pressure atomizers	Centrifugal energy used for rotary atomizers	Gaseous energy used for twin-fluid or blast atomizers
Fan-shaped sprays		Low pressure
Fan spray nozzles		0.2–2 p.s.i.
(single orifice)		Gas/liquid ratio,
25–125 p.s.i.		15–25 lb./lb.
Impact nozzles	Spinning cups	
Impinging jet nozzles	20–100 ft./sec.	
20–140 p.s.i.		
Solid surface impact nozzles		Medium pressure
Up to 400 p.s.i.	Spinning disks	2–20 p.s.i.
Deflector nozzles	100–600 ft./sec.	Gas/liquid ratio,
100 p.s.i.	Flat disks	0.2–1 lb./lb.
	Saucer-shaped disks	
Conical-shaped sprays		
Swirl spray nozzles	Radial-vaned disks	
40–100 p.s.i.		
Hollow cone	Multiple disks	
Drowned cone		High-pressure
Divergent pintle nozzles		Above 20 p.s.i.
		Gas/liquid ratio,
20–1000 p.s.i.		0.2–5 lb./lb

atomizers is even more confusing. The following terms are all employed (as indicated in parentheses) to indicate the same thing, namely, a swirl spray pressure nozzle (as in Table II): pressure jet atomizers (combustion engineers), swirl atomizers or rotational sprayers (gas turbine engineers), centrifugal nozzle (United States), injector fuel valve (marine engineers), and hydraulic cone spray nozzle (agricultural engineers).

Even more confusion may arise in the future because modern atomizers will combine two or even all three sources of energy. For example, a swirl spray nozzle is combined with a medium-pressure twin-fluid atomizer to improve the atomization of viscous liquids; a spinning cup or disk is combined with a large volume of low-pressure air to give projection velocity and further disintegration; or a pressure atomizer working at low pressure to give large drops is combined with low-pressure air to give control of direction of the drops. This last combination is particularly applicable to open-air spraying where small drops are not required. A medium-pressure blast atomizer is combined with a large-volume high-velocity air jet to give further disintegration of small drops for the purpose of producing aerosols.

B. THE MECHANICS OF PRODUCING A LIQUID SHEET

One of the primary functions of an atomizer, often ignored by the engineering designer, is that it should form the liquid into a sheet or a large number of small liquid jets. To achieve this a variety of mechanical means have been devised.

1. *Production of the Sheet by Pressure*

The need for the simplest equipment has made pressure atomizers the most attractive. The design suffers from low flexibility or turndown ratio because the flow rate varies with the square root of the pressure. In special applications pressures up to 8000 p.s.i. have been employed to improve the ratio, but in commercial spraying equipment 350 p.s.i. is usually considered an economic limit, with 1000 p.s.i. as a maximum. Single-hole nozzles (Fig. 2*a*,*b*,*c*) and impinging jets (Fig. 2*d*,*g*) at pressures up to 125 p.s.i. have shown their usefulness for small-volume spraying. Impact nozzles (Fig. 2*e*,*f*) using solid surfaces have the virtue of great simplicity and mechanical robustness and are useful for irrigation purposes at pressures up to 400 p.s.i. For long-range, large-volume sprinkling the simple orifice at a high pressure can achieve what is required, and swirl spray nozzles (Fig. 6) producing conical sprays ranging from narrow-angle drowned sprays up to wide-angle hollow sprays can satisfy most of the larger-volume spraying.

The basic function of pressure nozzles is to convert the pressure energy in the bulk liquid into kinetic energy in the form of a thin sheet of liquid moving at high velocity. The stability of the sheet is influenced by the dynamic properties of the liquid and the gaseous medium into which it is discharged. Thus the performance of pressure nozzles depends upon these factors, and their design must be related to them.

a. Fan-Shaped Sheets: Single-hole fan spray nozzles. To cause a fluid to flow into a flat sheet, either two streams are required to travel toward each other, in space or behind an orifice, or a liquid jet must

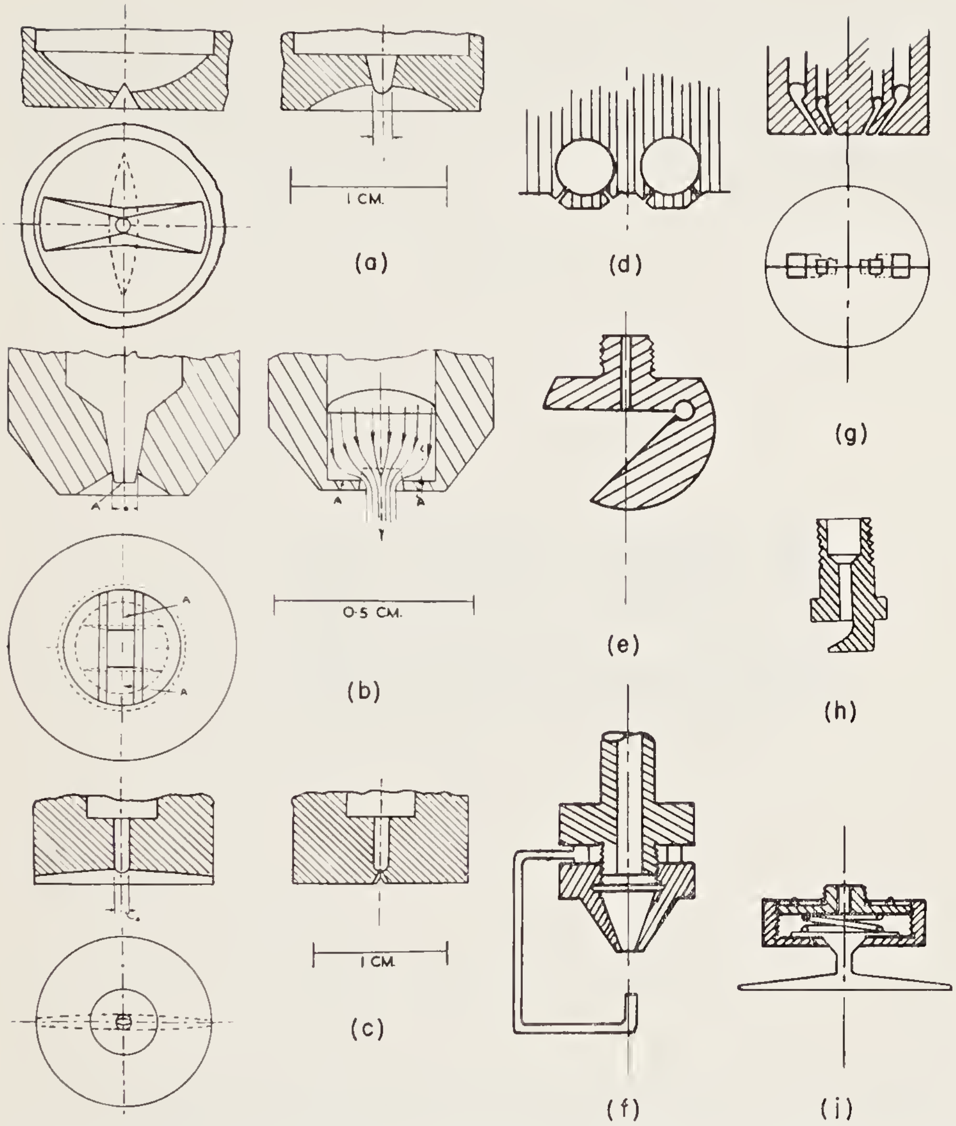


Fig. 2. Designs of pressure nozzles producing flat sheets: (*a*,*b*,*c*) types of single-hole nozzles, (*d*,*e*) impinging jets, (*f*,*g*) impact nozzles, (*h*) deflector nozzle, (*i*) diaphragm nozzle.

be made to strike a smooth surface at a high angle of incidence. Where two jets of liquid are caused to strike each other at an angle, usually greater than 90°, a thin sheet is formed in a plane perpendicular to the plane of the jets and containing their bisector, as shown by Fig. 3*a*. Figure 3*b* illustrates the fully opposed jets at 180°, which will cause the liquid to spread out in a circular sheet. If a large part of such a circumferential slot is closed, the liquid behaves similarly and a sector, or fanlike sheet, is produced. If the diameter of a tube is large, it is not necessary for the liquid to be flowing initially in both directions, because it will do so at the curved slot orifice (Fig. 3*c*).

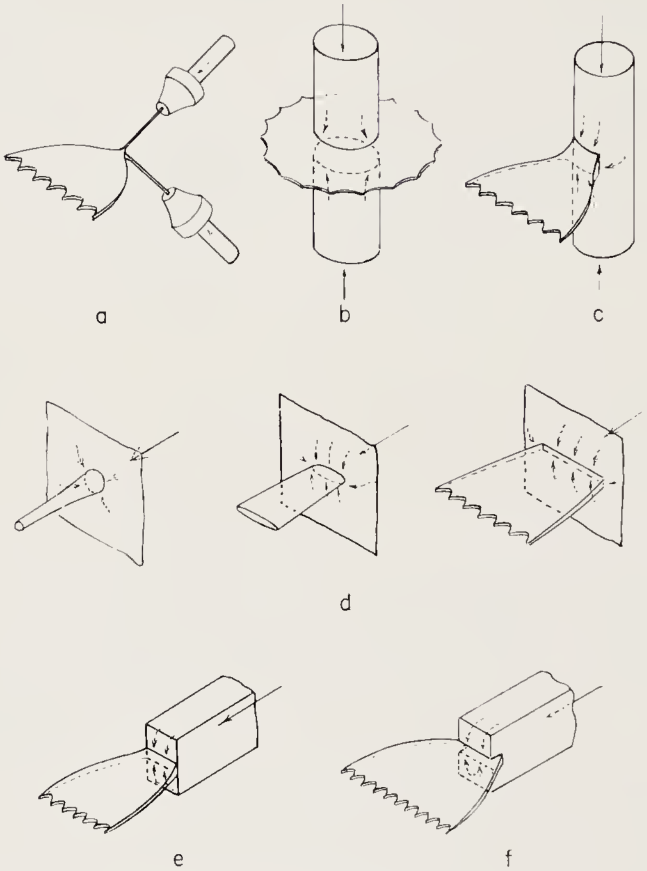


Fig. 3. Methods of producing flat liquid sheets: (*a*,*b*,*c*) opposing jets, (*d*) slot nozzle, (*e*,*f*) wedge nozzle.

If the boundary-flow-streamlines toward a circular orifice are curved so that flow occurs radially inward from all directions, they form a circular jet. When a circular orifice is distorted and made elliptical or eventually into a slot, the streamlines flow toward each other mainly from two directions, and thus the liquid issues as a sheet in the plane of the slot (Fig. 3*d*). If the slot is in a flat surface, the sheet has a small spray angle; and if the slot is of uniform width, the flow contributed by the ends will cause thickening of the rims of the sheet.

If a short slot or rectangular orifice is formed at the end of a rectangular tube (Fig. 3*e*), the streamlines at the edge are diminished so that the flow is now almost entirely in opposite directions and a sheet is produced having a greater angle. In this case the center of pressure is behind the rectangular orifice, and the edges of the sheet are restrained by the side walls of the rectangular tube. When the side walls are cut away, as in Fig. 3*f*, the edges of the sheet are not restrained and the divergence of the sheet is dependent only upon the pressure and the properties of the liquid. These flow characteristics are utilized in the various designs of fan spray nozzles. The shaped orifice of these nozzles is the functional part which controls not only the liquid approaching the orifice but also the geometry of the impinging streams behind it and the shape of the resulting sheet on its outside. The area coefficient of such nozzles is unity, the orifice being fully occupied by the liquid.

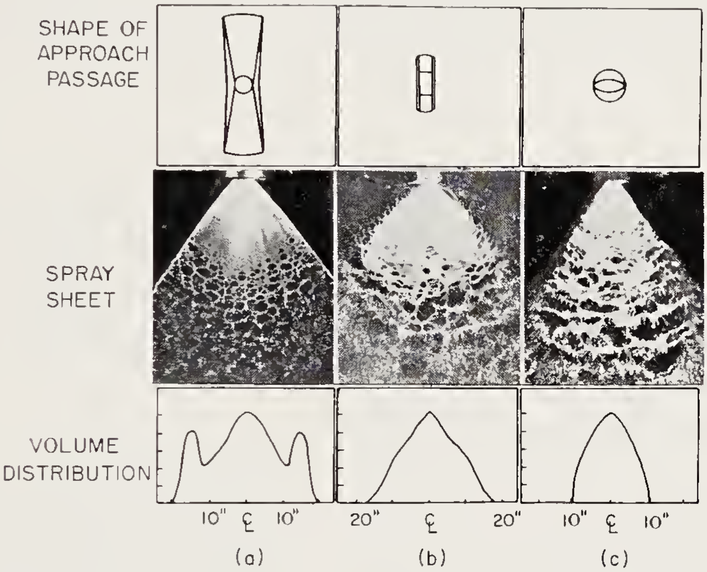


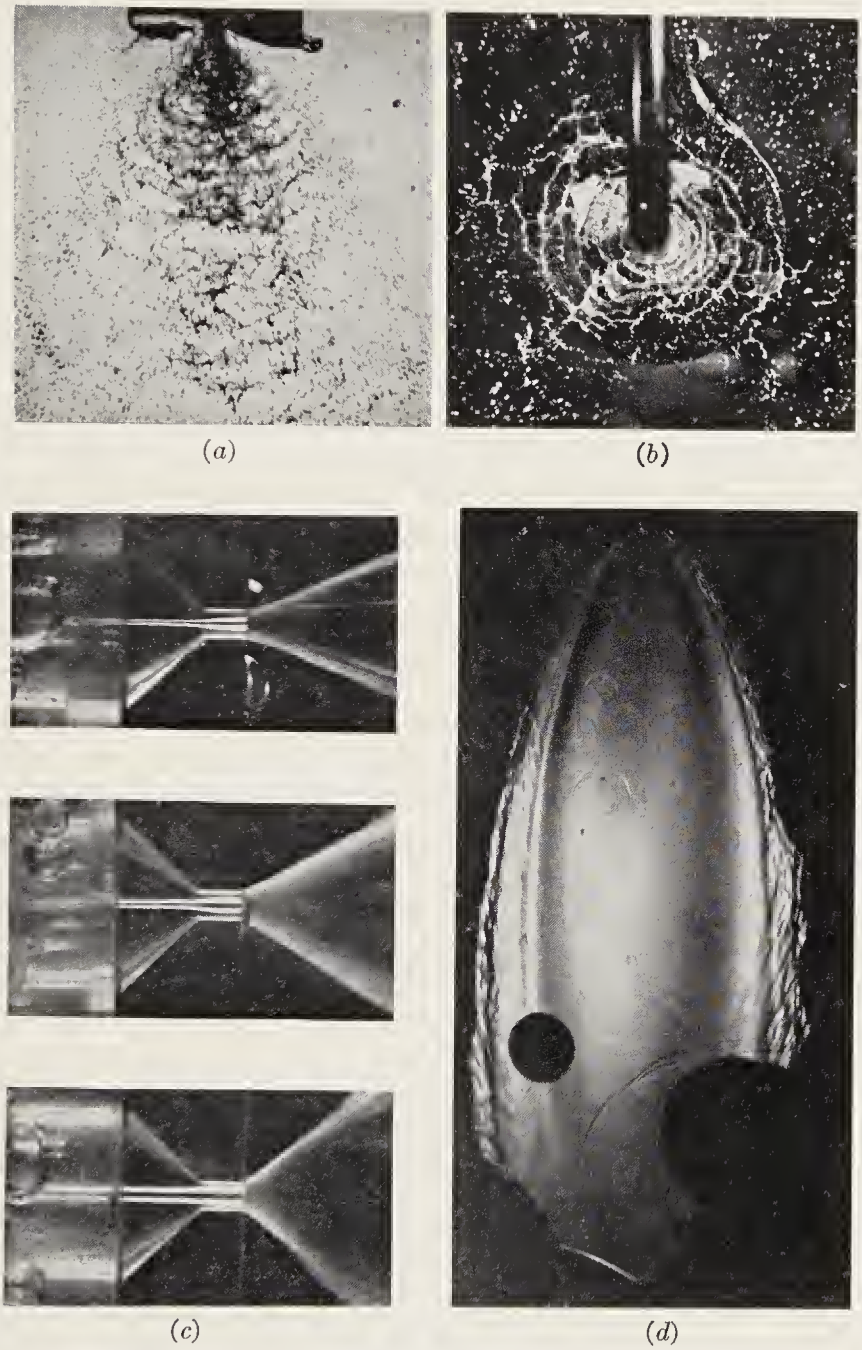
Fig. 4. Influence of design on spray pattern and dispersion from single-hole nozzles (as Fig. 2*a*,*b*,*c*).

There are a number of characteristic types of single-hole fan spray nozzles used in agriculture, some being shaped from metal with special tools and others molded in ceramic. Three characteristic types, each having an orifice of different shape, are illustrated in Fig. 2*a*,*b*,*c*, and

Fig. 4 shows the influence of the orifice shape on the spray pattern and deposition characteristic (12). The use of these nozzles is particularly advantageous in small-volume spraying, below 10 gal./hr., because the nozzles are simple and cheap and when damaged can be thrown away. Small swirl spray nozzles, on the other hand, are difficult to manufacture with high accuracy and, consequently, are much more expensive.

Impact nozzles (impinging jet nozzles). The principle on which the impinging jet nozzle operates is the same as that used in the single-hole fan spray nozzle except that jet impingement takes place outside the orifice. For the production of symmetrical and thin sheets it is essential that the two jets should be aligned as accurately as possible and that the liquid jets should be stable before impact. A stable jet can be obtained by selecting the correct L/D ratio for the orifice, and jet stability can also be improved if the turbulence in the jet is fully developed before it issues from the orifice. Turbulence can be obtained by adding a uniform roughness to the walls of the orifice during machining. This method of disintegration is commonly used where two fluids have to be mixed, as in the liquid-propellant rocket motor. It also finds application in fire fighting, where a large number of pairs of jets can be arranged in line or radially on a common spray plate, so that any desired spray shape can be produced. Figure 2*d*,*g* shows two machined plates each containing a circular slot which, when assembled, produces the desired orifice. Figure 5*a* shows the development of the sheet from a 70° impinging jet atomizer where the plane of the jet is at an angle to the paper. A ruffled sheet of liquid is formed which disintegrates intermittently to form groups of drops. Waves appear to be propagated from the point of impingement, and the frequency of these waves increases with the jet velocity. Heidmann and Humphrey (44) showed that at a constant jet velocity the wave frequency increased with increasing jet diameter. The frequency of the waves may be as high as 4000 cycles/second.

Solid surface impact and deflector nozzles. Where a cylindrical jet is made to impinge at high velocity on a smooth surface disposed close to the jet orifice, which surface may be inclined to the jet, the momentum in the liquid causes the streamlines to spread out and lie against the surface, so that a sheet is formed having an angle of spread depending upon the angle of inclination to the jet. If the solid surface is made just big enough to effect the deflection, the un-



(*a*) (*b*)

(*c*) (*d*)

Fig. 5. (*a*) Sheet from an impinging jet nozzle. (*b*) Sheet from an impact nozzle. (*c*) Air core development and spray angle variation with A_s: (top) 47°, $A_s = 22$ mm.^{2}; (center) 61°, $A_s = 11$ mm.^{2}; (bottom) 85°, $A_s = 2.2$ mm.^{2}. (*d*) Ripples from point sources on a fan spray sheet *in vacuo* (photograph by specular reflection).

supported sheet leaves the periphery before it disintegrates. Two designs of such nozzles are shown in Fig. 2*e*,*f*. Figure 5*b* shows the sheet after impact in design (*f*) spreading out in all directions in a series of circular waves from the point of impact.

If the jet is not allowed to form before contacting the deflecting surface, the nozzle is a deflector nozzle, and the mechanism of disintegration is similar to a solid surface impact nozzle. Figure 2*h* is a deflector nozzle similar in design to type (*e*). Figure 2*j* is a nozzle employing a diaphragm where the liquid is guided along the stem and issues from a conical solid surface.

b. Conical Sheets: Divergent pintle nozzles. When a liquid is caused to flow through a narrow divergent annular orifice or around a pintle against a divergent surface on the end of the pintle, a conical sheet of liquid is produced, where the liquid is flowing in radial lines. The angle of the cone and the root thickness of the sheet can be controlled by the divergence of the spreading surface and the width of the annulus. When design calls for very small outputs, this method is not so favorable because of difficulties in attaining an accurate annulus.

If the pintle or valve is mounted elastically, such as by means of a spring, so that the annular orifice may vary according to pressure (Fig. 6*a*), then the valve may oscillate at quite high frequencies, up to 1000 cycles/sec., so that the liquid delivery is impulsive. The flow characteristics of such a nozzle have been studied by Sliepcevich *et al.* (92) at pressures up to 3000 p.s.i. They noted that the best atomization appeared to occur when the frequency was high. The drop size dispersion of such nozzles, however, is wider than with a normal free-flowing conical sheet (36).

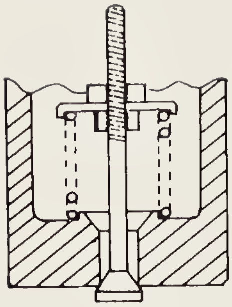
Swirl spray nozzles. When a liquid is caused to emerge from an orifice with a tangential velocity component, resulting from its path through one or more tangential or helical passages before the orifice, a conical sheet is also produced. In such swirl spray nozzles the rotational velocity is sufficiently high to cause the formation of an air core in the orifice and in the swirl chamber before the orifice. In these circumstances the area coefficient of the nozzle is very low. Figure 8 shows a schematic diagram of the flow and the important dimensions which determine the performance of such nozzles.

Figure 5*c* shows the air core formation in a transparent swirl nozzle with change in the degree of swirl. Viscous drag at the surface of the swirl chamber retards the rotating liquid, which is unable to remain in a circular path against the radial pressure balancing the centrifugal action. Consequently, a current can be set up directed toward the orifice through the surface boundary layer (102). Even for liquids of low viscosity, such as water, a considerable proportion of the flow can reach the orifice by way of this boundary layer.

The form of the conical sheet depends upon the working pressure and passes through a number of stages as the pressure is increased. At low pressures the liquid first forms a closed bubble. With increase of pressure the bubble opens to form a hollow cone, and the region of disintegration moves away from the orifice. The pressure at which the bubble opens to a hollow cone depends upon the nozzle design and physical properties of the liquid, particularly on the viscosity and surface tension. As the pressure is further increased, the region of disintegration of the sheet moves back toward the orifice. A common practice, to obtain drowned sprays, where the spray cone is not hollow, is to allow a further jet of liquid to enter the swirl chamber centrally from its base so that the air core and the hollow sheet are destroyed.

The manufacture of swirl spray nozzles requires great precision, and many abortive attempts have been made to find cheap methods of production which often result in a marked loss of performance. This is particularly true of small nozzles with low flow number. Figure 6 shows a variety of typical swirl spray nozzles. The swirl chamber can be either conical or cylindrical, but almost invariably the orifice consists of a short cylindrical tube L/D less than 2. The greatest variation in the design occurs in the geometry of the inlet swirl ports. A swirl plate or plug is commonly used with symmetrical tangential grooves or holes. One of the simplest designs in agriculture consists of a combination of a swirl plate and orifice plate in one body, as shown in Fig. 6*b*,*c*,*d*.

One form of plate design is the Long Ashton Spray Nozzle (52) (Fig. 6*b*), which was evolved as a result of efforts to standardize the design of a swirl spray nozzle of large throughput for orchard spraying and general purposes. It consists of a body with coupling nut and three interchangeable parts, the orifice disk, swirl plate, and washer, which are made to several standard dimensions. Disks are made with several orifice diameters, swirl plates have 2, 4, or 6 slots inclined to the axis and/or central holes, and washers have various thicknesses to allow variation of swirl chamber depth. By selecting various and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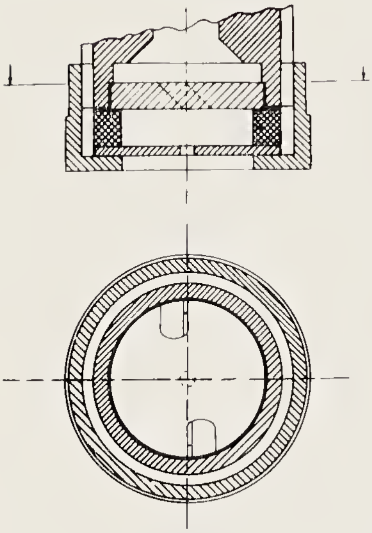
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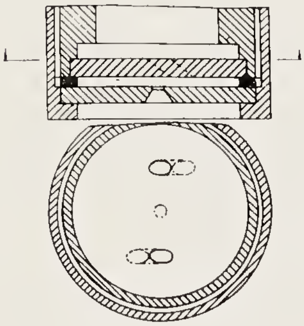
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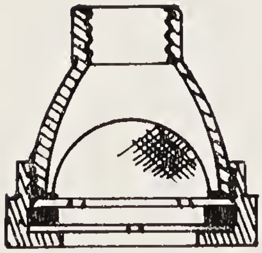
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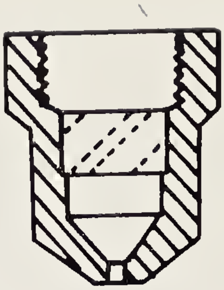
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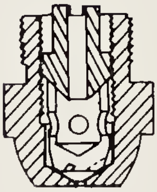
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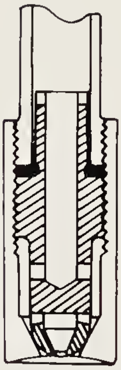
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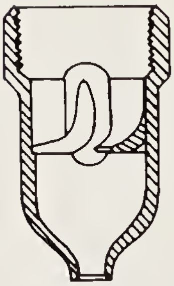
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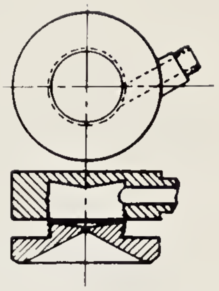
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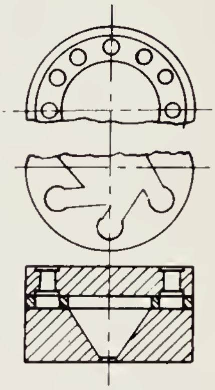
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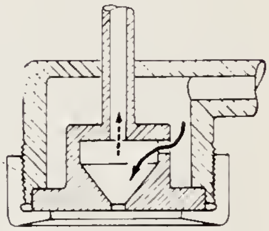
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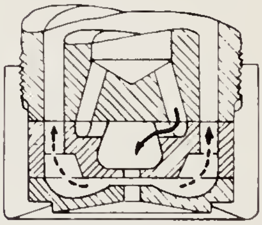
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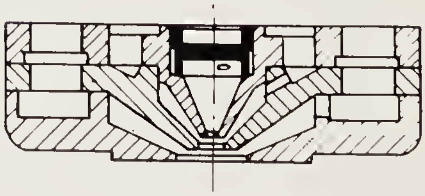

(m)

correct combinations of these parts and the spraying pressure, useful variations can be achieved in output, spray pattern, range, and drop size. Figure 7 shows a number of microsecond flash photographs of the spray sheet from various combinations of parts with this nozzle.

The simple swirl spray nozzle suffers from inflexibility of turndown because the discharge varies approximately as the square root of the pressure. Greater turndown ratio can be obtained with the spill type or range control atomizer. In these some of the liquid entering the swirl chamber is returned or spilled back, either through a controlled central return path (Fig. 6*k*) or by removing part of the boundary flow at the entry to the nozzle (Fig. 6*l*). By spill control, at a constant supply pressure, the turndown ratio can be increased to 20:1.

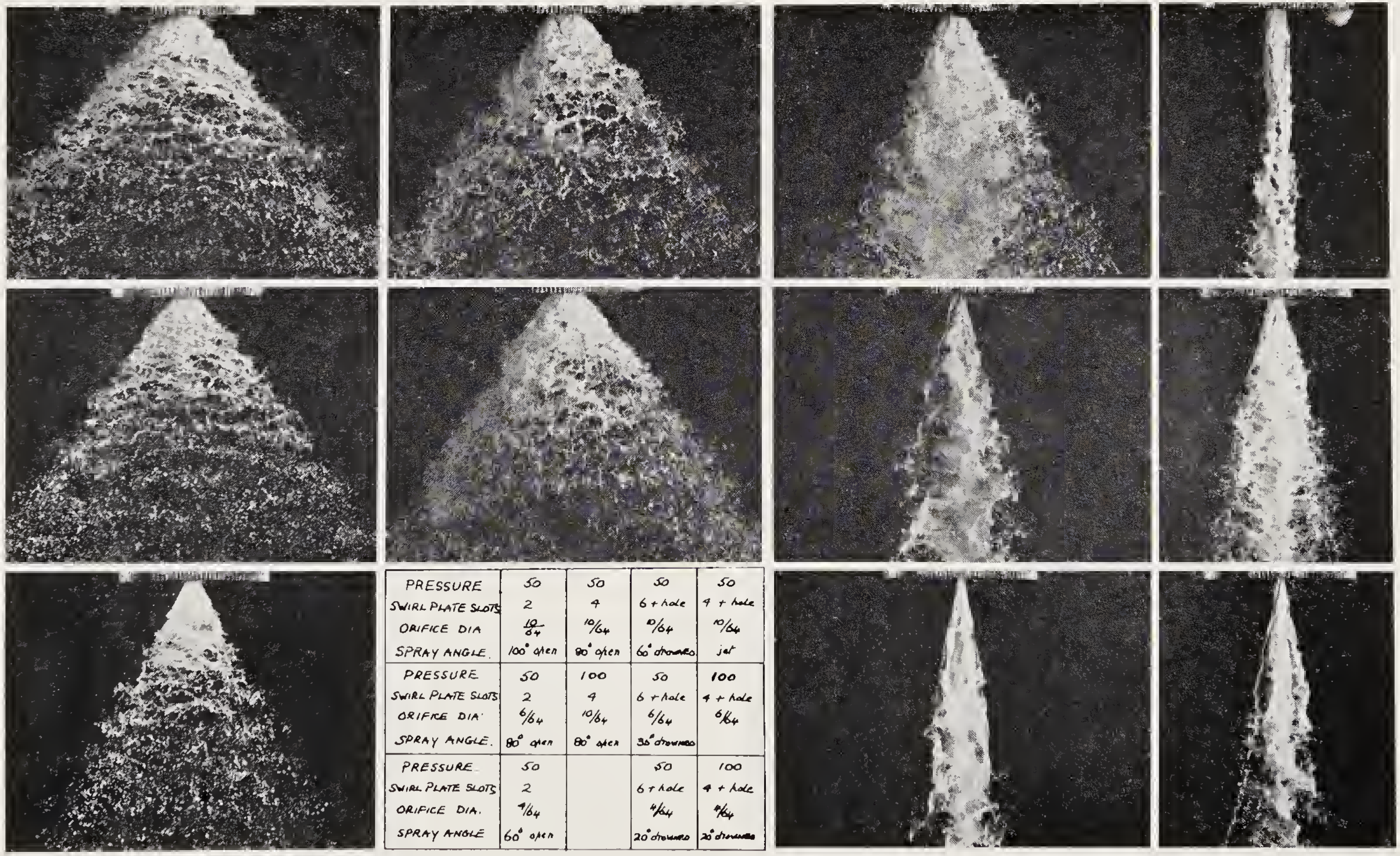
Another method of control is the expanding slot principle which, by adding a metering resistance, compensates to an extent the square law effect. A further method, commonly used for liquid fuel burning, is the duplex or triplex atomizer, where two or more nozzles are housed concentrically and are independently controlled (a duplex impinging jet atomizer is shown in Fig. 2*g* and a triplex swirl atomizer in Fig. 6*m*) so that one produces a pilot spray and the other a main spray (106). Although spill devices improve the range over which atomization is satisfactory, they do not always solve the problem of maintaining an approximately constant drop size as the flow rate is increased.

Dimensional relations for the design of swirl spray nozzles. The apparent simplicity of the swirl spray nozzle has led engineers for a long time to suppose that they could design such a unit by merely recognizing its general shape. It has taken many years and the work of many investigators to derive the necessary design data to obtain a reasonably efficient solution for a nozzle for a particular liquid, pressure, spray angle, and drop size (8,9,15,42,43,47,73,78,93,95,100,102, 106,108).

Swirl spray nozzles differ from other types of pressure atomizers in that, in addition to the orifice diameter, other dimensions have a great effect on the discharge coefficient and the spray angle, both of

---

Fig. 6. Designs of pressure nozzles producing conical sheets: (*a*) vibrating valve nozzle, (*b,c,d*) interchangeable plate type, (*e*) unitary type, (*f*) plug type (liquid fuel), (*g*) interchangeable unitary element, (*h*) unitary type (large volume, low pressure), (*i*) single swirl port type, (*j*) swirl plate type (liquid fuel), (*k*) central spill type, (*l*) side spill type, (*m*) triplex type.



PRESSURE	50	50	50	50
SWIRL PLATE SLOTS	2	4	6 + hole	4 + hole
ORIFICE DIA	10/64	10/64	10/64	10/64
SPRAY ANGLE.	100° open	80° open	60° drowned	jet
PRESSURE	50	100	50	100
SWIRL PLATE SLOTS	2	4	6 + hole	4 + hole
ORIFICE DIA	6/64	10/64	6/64	6/64
SPRAY ANGLE.	80° open	80° open	30° drowned	
PRESSURE	50		50	100
SWIRL PLATE SLOTS	2		6 + hole	4 + hole
ORIFICE DIA.	4/64		4/64	4/64
SPRAY ANGLE	60° open		20° drowned	20° drowned

Fig. 7. Conical sprays from typical combinations of components of Long Ashton agricultural spray nozzles.

which may be varied over wide limits. The purpose of design theories is to relate nozzle dimensions to discharge coefficient and spray angle, since the performance of the atomizer depends upon these flow characteristics. Several design theories to determine these flow characteristics have been proposed. According to ideal flow analysis the discharge coefficient, C'_Q, and the spray angle, θ, should depend only on the geometry of the nozzle expressed by the dimensionless number Δ:

$$\Delta = A_s/D_2D_3 \qquad \text{or} \qquad \Delta = A_s/D_2D_M \tag{2}$$

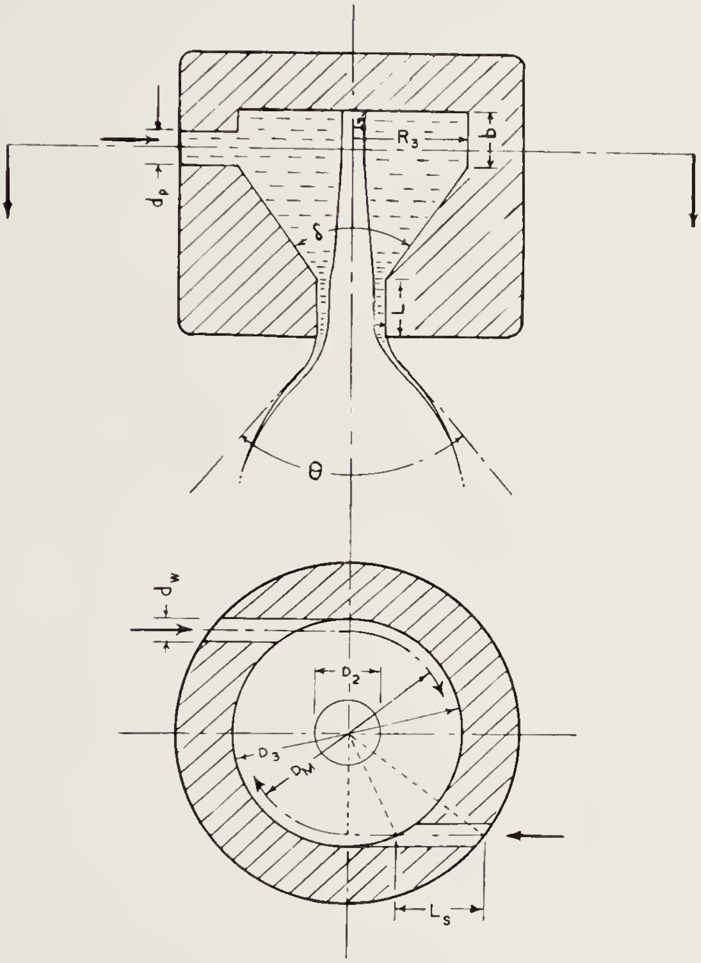


Fig. 8. Schematic diagram of swirl spray nozzles.

A_s is the total cross-sectional area of inlet swirl ports; D_2 is the orifice diameter; D_3 is the swirl chamber diameter; D_M is the diameter of the swirl chamber vortex (see Fig. 8).

Figure 9 shows these relationships according to the ideal frictionless theories of different authors. It is observed that although there is considerable variance among them due to different simplifying assumptions, the higher Δ, the lower the spray angle and the higher the discharge coefficient. These deductions hold in practice, but it is found that the flow characteristics depend also on other dimensions of the nozzle; in particular, the ratios of the orifice length to the diameter (L/D_2) and of the swirl chamber vortex to the orifice diameter (D_M/D_2), as well as on liquid properties, especially viscosity.

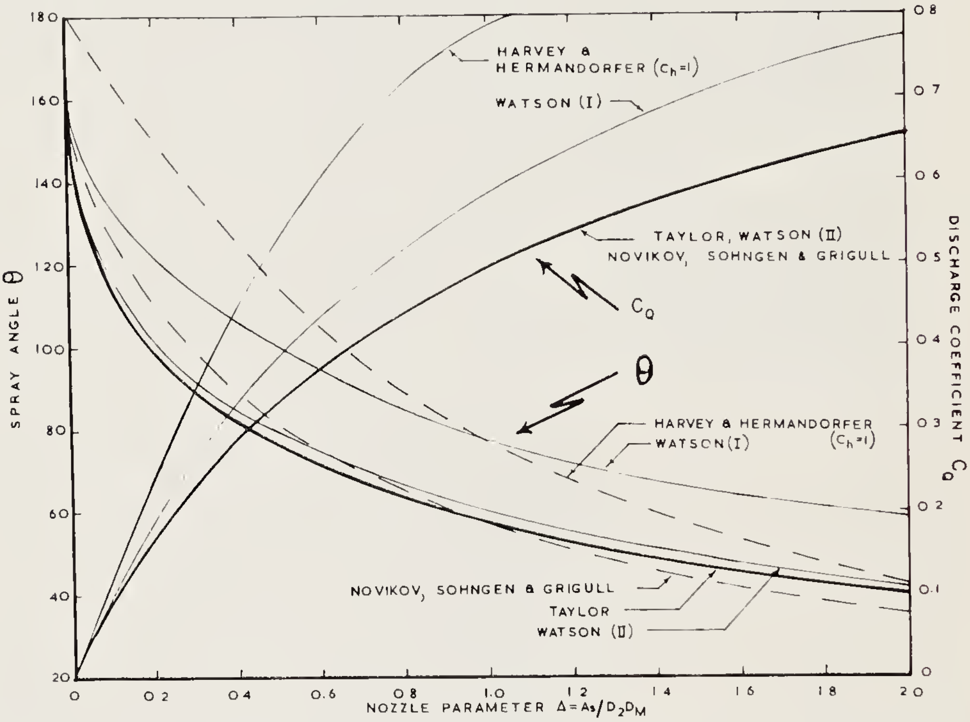


Fig. 9. Relationship between swirl nozzle parameters and flow characteristics according to various ideal theories.

It has been shown (15,43) that for a nozzle of given Δ the discharge coefficient increases with increasing D_M/D_2 and with diminishing L/D_2. The spray angle increases with both diminishing D_M/D_2 and L/D_2, for there is a unique relationship between discharge coefficient

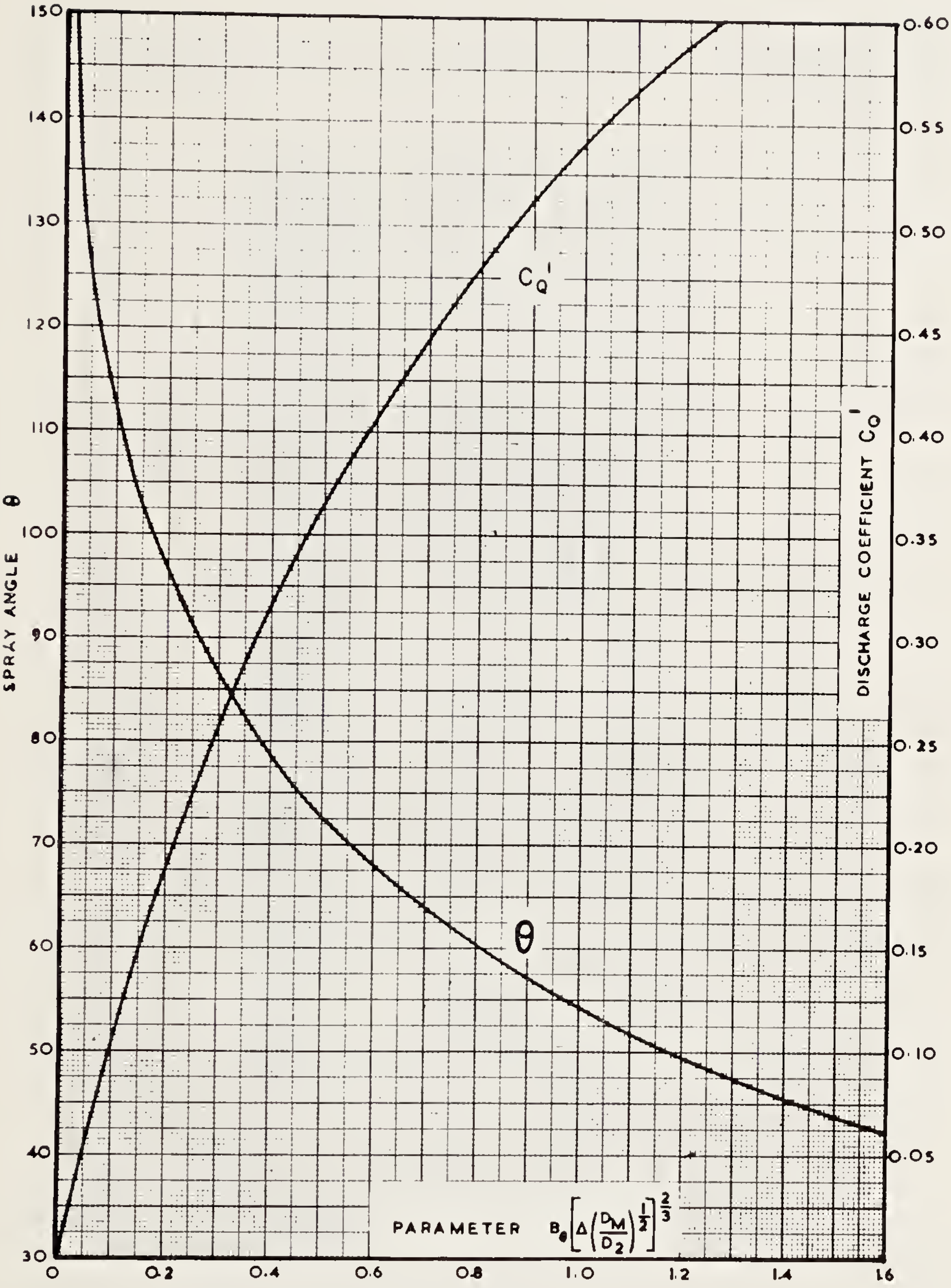


Fig. 10. Relationship between effective swirl nozzle parameter and flow variables.

and spray angle which is determined only by the ratio L/D_2. These phenomena have been satisfactorily explained by examining the frictional decay of the vortex in the swirl chamber.

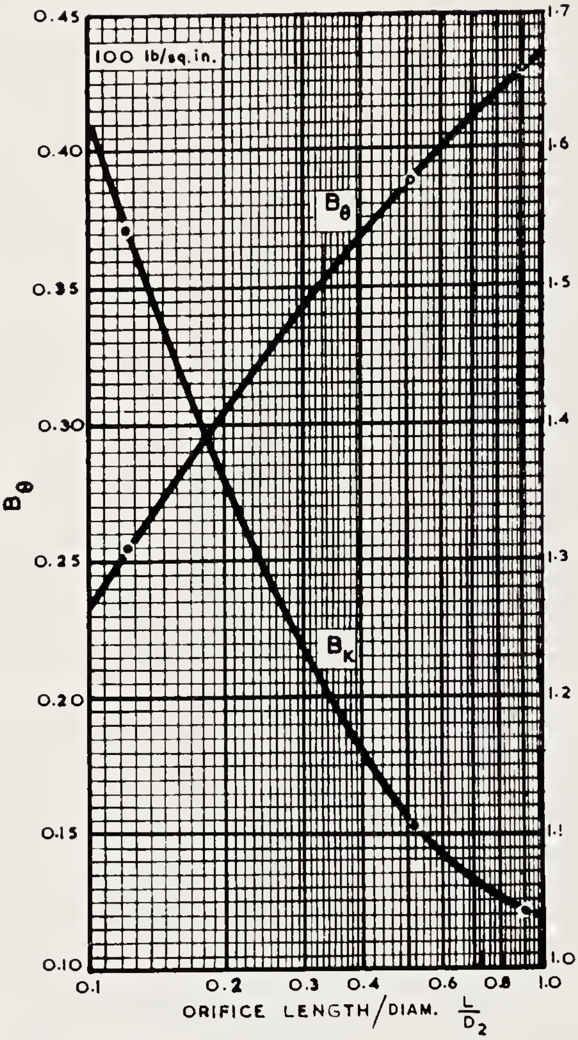


Fig. 11. Correction factors for orifice L/D_2 effects.

The empirical law for the decay of the tangential velocity of a vortex, VR^n = constant, expresses the well-established frictional effect in the vortical motion of a real fluid. When it is applied to the flow through a swirl spray nozzle the frictionless parameter, Δ is found to be only a special form of the more general expression

$$\Delta(D_M/D_2)^{1-n} \tag{3}$$

It has been found (43) that by selecting an appropriate value for n for each nozzle ($n = 0.4$–0.6), the measured flow characteristics for water of various designs coincide and the results of many investigators can be correlated using this parameter, n being assumed to have the value for which the measured flow characteristics of nozzles of varying D_M/D_2 are found to fall on a single curve, which suggests that the same frictional index n holds for all nozzles of one particular design using the same liquid.

Experiment has shown that the flow characteristics with low viscosities can be predicted using the above parameter if simple empirical corrections are made. For design purposes the actual spray angle is directly obtained from the curve in Fig. 10 where the nozzle parameter is modified to

$$B_\theta[\Delta(D_M/D_2)^{1/2}]^{2/2} \tag{4}$$

This modified parameter predicts an apparent discharge coefficient, C_Q', which is linearly related to the true discharge coefficient, C_Q, by

$$C_Q = B_K \times C_Q' - 0.05 \tag{5}$$

The values of the correction factors B_θ and B_K, for the effects of L/D_2 on the spray angle and the discharge coefficiency, respectively, are given in Fig. 11.

Decrease of Reynolds number at the orifice (i.e., diminishing the orifice diameter or increasing the viscosity) leads to an increase in discharge coefficient and to a decrease in spray angle. This is again an effect of friction which, in general, causes a reduction in the tangential velocity, thus decreasing the air core diameter, with a consequent increase in discharge coefficient and decrease of spray angle. At the same time, there are losses in the other velocity components of the liquid, tending to reduce the discharge. This explains why, beyond a certain limit, the increase of discharge with liquid viscosity reaches a maximum, after which the discharge is reduced. Under these conditions the air core disappears and atomization is poor.

The characteristics of swirl spray nozzles are summarized in Table III.

c. *Energy Requirements for Pressure Nozzles.* Monk (69) estimated the viscous energy dissipation required when considering the ideal steady reduction in the diameter of a volume of liquid to a small filament. He showed that the energy required was very high, indicating

TABLE III

Summary of Characteristics of Swirl Spray Nozzles

At constant liquid pressure
  Flow $\propto$ (nozzle diameter)^2
  SMD $\propto$ (nozzle diameter)$^{2/3}$
With constant nozzle size
  Flow increase $\propto$ (pressure)$^{1/2}$
  SMD reduces $\propto$ (pressure)$^{1/3}$

the extreme difficulty involved in reducing liquids to filaments below 10 μ. For example, the minimum viscous energy dissipated upon reducing 1 cc./sec. (or 8 lb./hr. $\rho_L = 1$) of liquid of 1 cp. viscosity to a single filament of 5 μ diameter was 9870 ft.-lb.; an impossibly high figure. For a single filament of 20 μ diameter 40 ft.-lb./cc./sec. was required, which corresponds to 3000 ft.-lb./Imp. gal./min. or 0.09 hp./gal. For a single filament this energy corresponds to a liquid pressure of 7900 p.s.i. In practice, hundreds of filaments are produced simultaneously per second so that the pressure actually required is proportionally reduced and the mechanism of producing threads is from a sheet of greater surface energy than is contained in the filaments or drops (see Section III.B). The energy required for pressure nozzles is given by

$$E(\text{ft.-lb./min.}) = 23.0Q\Delta p \tag{6}$$

where Q is the flow rate in Imp. gal./min. and Δp is the pressure drop in p.s.i. The horsepower required is given by

$$\text{hp.} = 7.0Q\Delta p \times 10^{-4} \tag{7}$$

from which it will be seen that a theoretical horsepower requirement for a flow rate of 1 Imp. gal./min. at 100 p.s.i. is 0.07 hp., to which must be added hydraulic losses and prime mover inefficiencies.

2. Production of the Sheet by Centrifugal Force (Rotary Atomizers)

When the nature of the liquid is such that it will have a high viscosity, 40 cp. even when preheated, or where suspensions have to be sprayed, pressure atomizers cannot be used satisfactorily. Small channels become blocked, and overcoming the frictional drag requires excessively high pressures, which invite pump troubles. Nozzle wear, due to corrosion or erosion, produces larger and larger drop sizes.

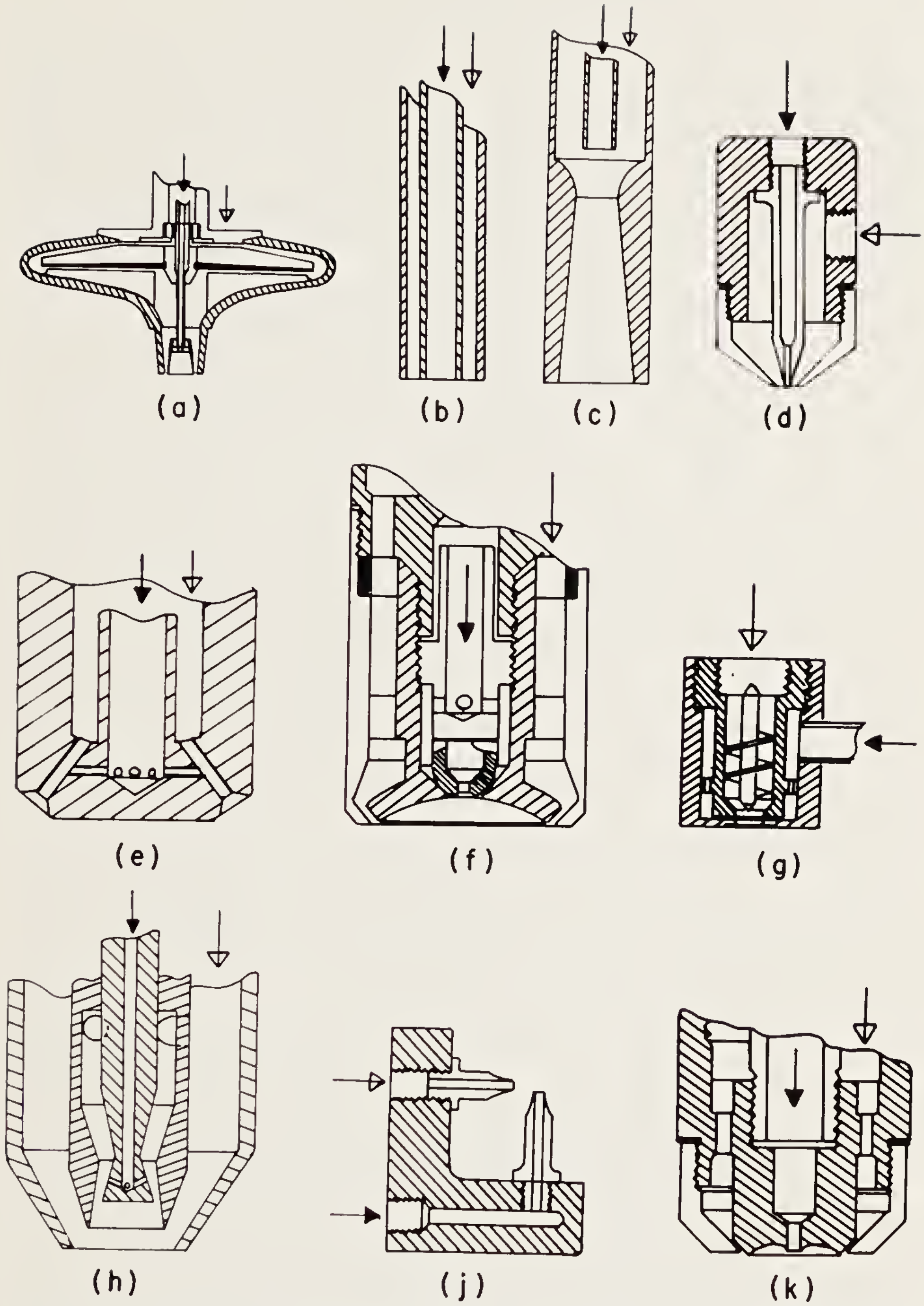


Fig. 12. Designs of twin-fluid atomizers: (*a*) spinning cup with fan, (*b*) injection type simple concentric tubes (external mixing), (*c*) injection type with Venturi (internal mixing), (*d*) injection type convergent annulus (external mixing), (*e*) multi-hole nozzle, (*f*) vortex cup with swirl spray pressure nozzle (external mixing), (*g*) internal swirl (internal mixing chamber type), (*h*) low-pressure vortex proportioning atomizer, (*j*) scent spray, (*k*) simple vortex cup (external mixing).

Although twin-fluid atomizers can handle such liquids and concentrates, they are more particularly useful for producing small drops. In such cases rotary atomizers are more promising. Here the liquid does not have to be forced through small openings, critical filtration is not necessary, and the drop size can be changed by variation of speed.

In rotary atomizers energy is imparted to the liquid by feeding it onto a rotating surface where it spreads out by centrifugal force, which overcomes the surface tension and viscous force of the liquid. The accelerating force is independent of the fluid quantity; thus the atomizer is extremely versatile. The rotating surface may take the form of a flat disk, cup, or slotted or shrouded impeller wheel. In practice, disk or cup diameters vary from about 1 in. up to 18 in., and large diameter disks may be rotated at 12,000 r.p.m. and have capacities of more than 5 tons/hr. So far, such large atomizers have had little application in agriculture. The spinning surface is more often of 1–6 in. diameter and surrounded by a mild air stream to give the drops direction or to cause further disintegration. Air stream velocities of 100–300 ft./sec. (3–30 in. H_2O) may be employed, in which case the energy in the liquid is small in comparison with that contained in the gas. The air surrounding the cup usually issues from an annulus of small area, and the capacity of the atomizer can be changed by simple adjustment of the annulus area. In this case the spinning element is employed merely as a device for producing and directing a liquid sheet, and the device should be classified as a low-pressure twin-fluid atomizer (see Section IV).

The spinning surface may be rotated by using some of the energy of the air itself, or it may be driven by mechanical means. With mechanical drive it is usually convenient to build the cup or disk on the shaft of the fan impeller (Fig. 12*a*), but this limits the peripheral velocity of the cup, usually below 50 ft./sec. Where the cup can be driven directly by an air turbine, wheel peripheral velocities of 600 ft./sec. can be achieved.

The velocity of the liquid flowing from the center of a rotating surface can be resolved at any point into a tangential and a radial component. With a frictionless liquid the tangential and radial velocities are equal, that is, at the periphery equal to πDN. The resultant velocity is, therefore, $(2\pi DN)^{1/2}$ and the maximum energy to be imparted to the liquid is $m(\pi DN)^2$. The theoretical power required to produce this energy is

$$\text{hp.} = 42.5m(DN)^2 \times 10^{-12} \tag{8}$$

where m is the mass flow rate in lb./hr.; N is the rotor speed in r.p.m.; and D is the rotor diameter in ft. Figure 13 shows the theoretical power required per unit mass flow rate as a function of disk diameter and r.p.m.

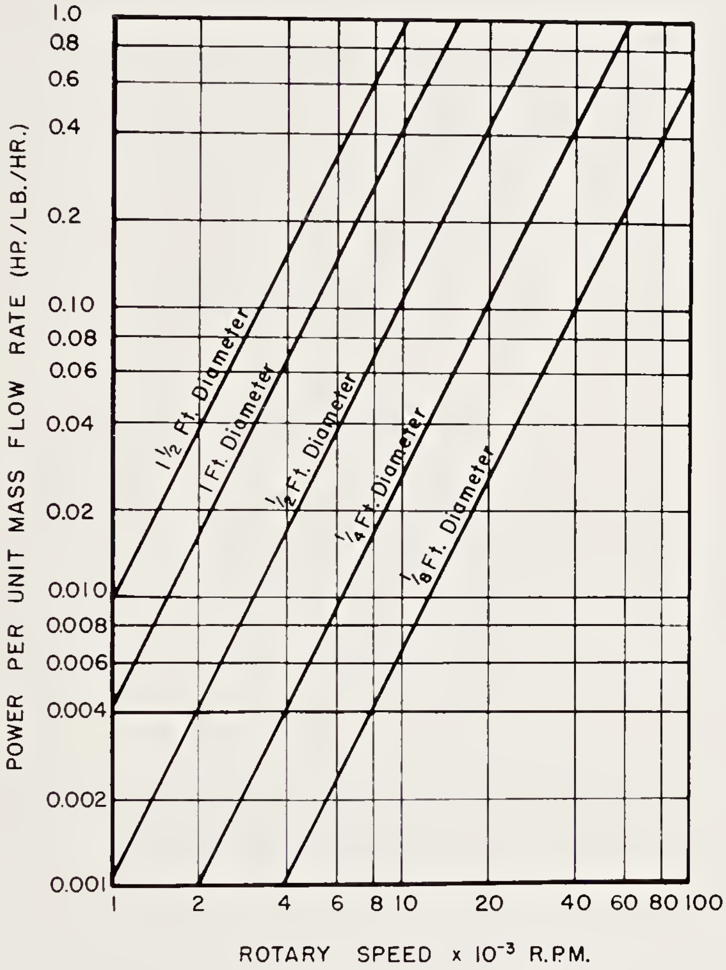


Fig. 13. Theoretical power requirement above no-load conditions for various diameter disks and speeds.

Under normal operating conditions the radial velocity is always very much less than the tangential velocity, so that the drops are centrifuged off the disk almost tangential to the periphery, about 1–2° to the tangent. Only at excessively large flow rates can large radial velocities occur.

At low velocities and feed rates the tangential velocity of the liquid leaving the disk can be nearly equal to the peripheral velocity, but flat disks are unsuitable for efficiently atomizing liquids at practical flow rates because they cannot impart the maximum tangential velocity due to slip between the surface and the liquid.

Slip can be reduced by shaping the disk as a cup or saucer and can be eliminated by constructing the rotor with vertical radial vanes so that the liquid is forced against them. Although this design is more efficient than a cup or saucer, it is more complex and more difficult to balance dynamically. The addition of vanes also increases the amount of air moved by the disk, which behaves as a fan impeller. Thus the power requirement may rise very steeply.

The conditions for severe slip for saucers are found to occur when the value of the relation

$$m/\eta\pi D > 1440 \tag{9}$$

where m is the mass flow rate in lb./hr.; η is the absolute viscosity in centipoise; and D is the disk diameter in ft.

Whereas the wetted perimeter for a disk or cup is fixed by the diameter, the wetted perimeter of a vaned disk can be increased by increasing the number and height of the vanes. This method is limited, however, because of the increasing difficulty of wetting the whole vane height.

The common dimensions of vaned rotating disks are: 8–24 vanes varying from $^1/_4$ to 1 in. high; disk diameters of 2–8 in.; speeds of 5000–30,000 r.p.m.; and feed rates of 12–3000 lb./hr.

3. Production of the Sheet by Air Blast (Twin-Fluid Atomizers)

In Table II twin-fluid atomizers are divided into three groups, according to the pressure of the air with which they operate:

(*1*) Those operating with low-pressure air, 5.5–56 in. H_2O, velocity 150–470 ft./sec., and which usually employ a high ratio of air/liquid, namely, 5–25 lb./lb., or 70–350 cu. ft. free air at 100°F./lb.

(*2*) Those which operate with air at medium pressures, between 2 and 20 p.s.i. or air velocities of 500–1000 ft./sec., where the air/

liquid ratio is usually 0.2–5 lb./lb., or 2.8–70 cu. ft. free air at 100°F./lb.

(*3*) Those which operate at high pressures, 20–150 p.s.i., and where velocities of 1000–2000 ft./sec. can be attained.

This grouping is related to the method of providing the gaseous medium, that is, by fan, rotary blower, or reciprocating compressor, respectively.

Twin-fluid atomizers can also be divided into external or internal mixers according to whether the gas and liquid meet outside in the atmosphere or internally, within the body of the atomizer. In the external mixers the fluid pressures are independent of each other. In the internal type the gas and liquid pressures react upon each other; therefore an adjustment in the flow of one affects the flow of the other, which makes control of flow rate more difficult. A further division in type may be made according to Table IV.

TABLE IV
Types of Twin-Fluid Atomizers

External mixing (fluid pressures independent)	
Drooling type	Fluid flow in same direction
Shearing type	Fluids meeting at an angle
Internal mixing (fluid pressures interacting)	
Chamber type	Premixing in chamber and exhausting through orifice or choke
Venturi type	Premixing within and exhausting through convergent-divergent nozzle

All blast atomizers have a large turndown ratio and, moreover, in certain designs the mean drop size is found to change very little as the quantity of liquid is reduced for a given air mass flow in a given size atomizer. This condition is advantageous where the same drop size is required over a range of flow rates (see Fig. 44, curves C and D).

The earliest recorded atomizer using steam was devised in 1866, and it is still typical of many of those used today, particularly for small throughputs where an annulus of high velocity air or steam surrounds a solid jet of liquid. Such atomizers are commonly referred to as the injection type, and they are usually operated at medium or high pressure. Figure 12*b,c,d* shows some of the simplest designs.

When the liquid flow rate is high and the liquid jet diameter thus becomes large, the efficiency of applying the blast energy in this way is diminished. It is inefficient because (*1*) the gas stream impinges upon a solid jet of liquid of large diameter and (*2*) the direction of the air is the same as that of the liquid. The gas quickly accelerates the liquid particles to its own velocity, and little further shear can occur. One method of overcoming this difficulty with atomizers of large flow rate is either to use a multi-hole nozzle (Fig. 12*e*) or to film the liquid into a thin sheet before it meets the high velocity air stream. There are a number of ways of doing this. The liquid may be caused to form a sheet by passing it through a pressure nozzle, as the first stage, to produce a conical film from a swirl spray nozzle (Fig. 12*f*), or a flat sheet may be formed by ejecting the liquid through an annulus either outside or internally within the atomizer (Fig. 12*g*). A further method is to cause the liquid to become spread out on a surface by aerodynamic action (Fig. 12*k*). When the prefilming method is applied, it is found that atomizers of large output can have nearly as small a mean drop size for the same gas/liquid mass ratio as the smaller size atomizer.

With low pressure the amount of air used for disintegration is not very critical. In spraying applications it is usually required that all the air be given a foreward component of velocity to obtain range. Thus the energy used is higher than that employed in the medium- and high-pressure type atomizers. In some applications too great a forward component of velocity is avoided by rotating all or part of the air stream and thus avoiding rapid equalization of the fluid velocities. In some types partial automatic control of the air/liquid ratio is possible by controlling the liquid flow from the low pressure created either within an air vortex inside the atomizer, as in Fig. 12*c*, when the liquid delivery point is in the throat, or over the liquid jet, as in the scent-spray type (Fig. 12*j*). In the controlled vortex type (Fig. 12*h*) using low pressure, the vortex tube element surrounding the oil jet may be moved forward to reduce both of the air annuli simultaneously and this movement may be further linked with the movement of a liquid slot valve so that a fluid proportioning atomizer is produced.

The principle of the wide tip or "vortex cup" type was developed by Fraser (18). In this form the gas is made to issue from an annulus surrounding, but some distance away from, the central liquid orifice and the liquid is made to spread out into a sheet over a surrounding surface or cup by aerodynamic action (Fig. 12*f*,*k*).

Figure 14*a* is an isobaric diagram of the air outside one of these atomizers. Liquid enters the central supply tube, and the jet issues from the center of the cup depression at low velocity. Air flows through the surrounding annulus at high velocity. The diagram shows the

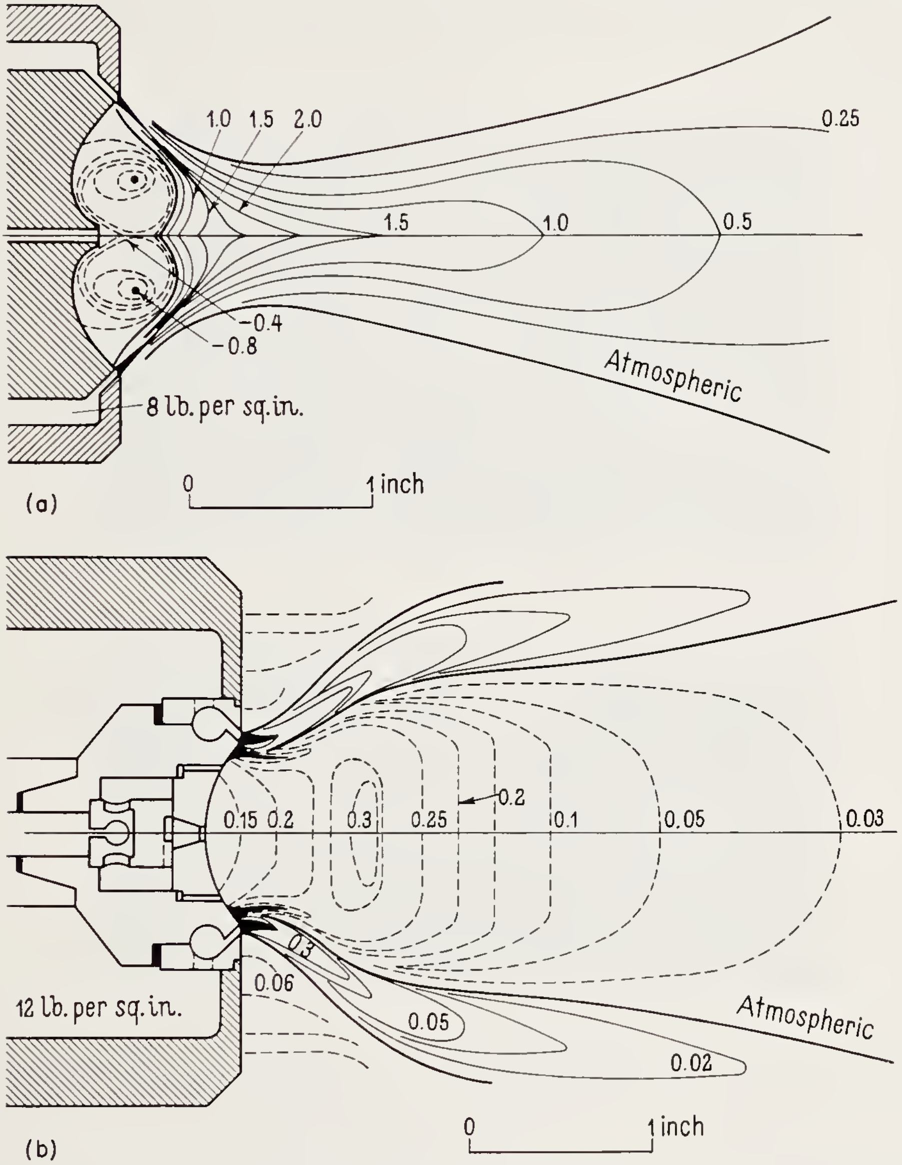
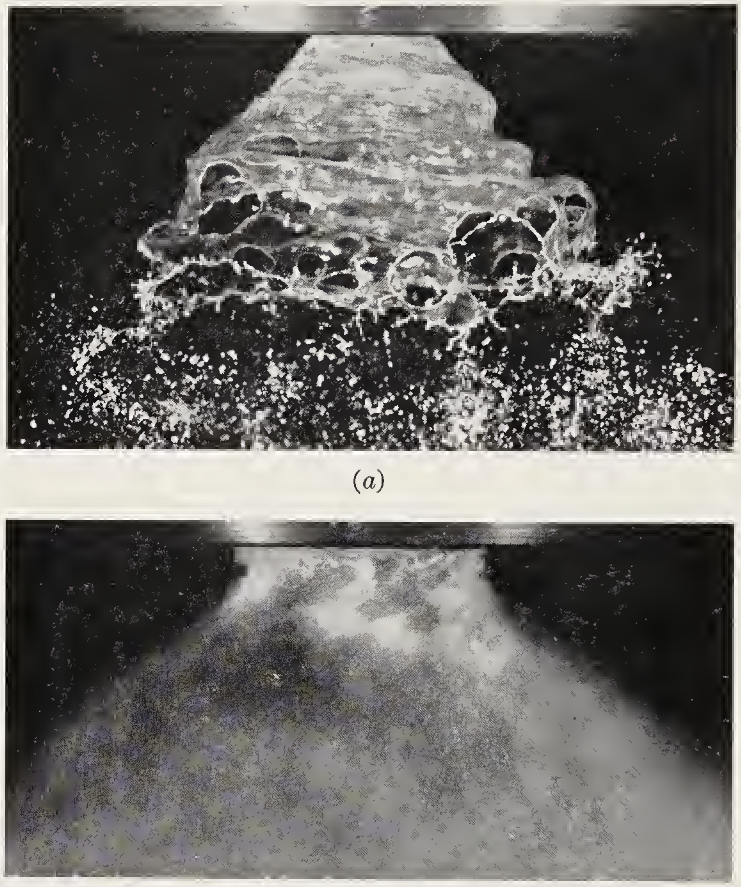

1.0
1.5
2.0
0.25
1.5
1.0
0.5
-0.4
-0.8
Atmospheric
8 lb. per sq. in.
0
1 inch
(a)
0.15
0.2
0.3
0.25
0.2
0.1
0.05
0.03
0.3
0.06
0.05
0.02
Atmospheric
12 lb. per sq. in.
0
1 inch
(b)

Fig. 14. (*a*) Isobar diagram of vortex cup atomizer (as Fig. 12*k*). (*b*) Isobar diagram of vortex cup atomizer with air swirl (as Fig. 12*f*).

distribution of pressure in the air outside the nozzle. The broken lines denote equal negative pressures. They show that a vortex ring is formed in the cup-shaped depression around the liquid orifice.

This vortex ring causes the air immediately around the orifice to be traveling in a direction opposite the liquid leaving the orifice. By this means the liquid is constrained to flow along the surface of the cup-shaped depression around the orifice and to spread outward as a sheet toward the periphery to meet the high velocity air stream from the annulus at right angles. The liquid sheet is then broken up by an interchange of momentum with the air stream and caused to flow toward the axis. The air pattern outside this nozzle can be likened to an inverted hollow conical sheet of air constraining a vortex ring against the face of the nozzle. Liquid is forced to rotate on the boundaries of the vortex ring until it can escape through the conical sheet



(*a*)

(*b*)

Fig. 15. Vortex cup atomizer (as Fig. 12*f*): (*a*) with water only at 100 lb./hr., (*b*) atomization with air at 14 p.s.i., circa 750 ft./sec.

of air itself and be propelled forward. Thus the time of contact between the liquid and the high velocity air during which atomization can occur is increased and the energy transfer improved.

Figure 14*b* shows a further development (19) where a swirl spray nozzle is placed centrally within the vortex cup. The air, in this case, before issuing from the annulus as a convergent stream is given a rotary motion by passing through swirl slots. The drop in pressure occurs in two stages: first across the swirl slots and then across the annulus. By adjustment of the annulus, therefore, the ratio of tangential to axial velocity can be varied and, thus, the angle of the spray. Figure 15 shows two photographs of the operation of this atomizer: (*a*) water at 100 lb./hr. flow rate from the swirl spray nozzle and (*b*) the increased atomization with air at 14 p.s.i.

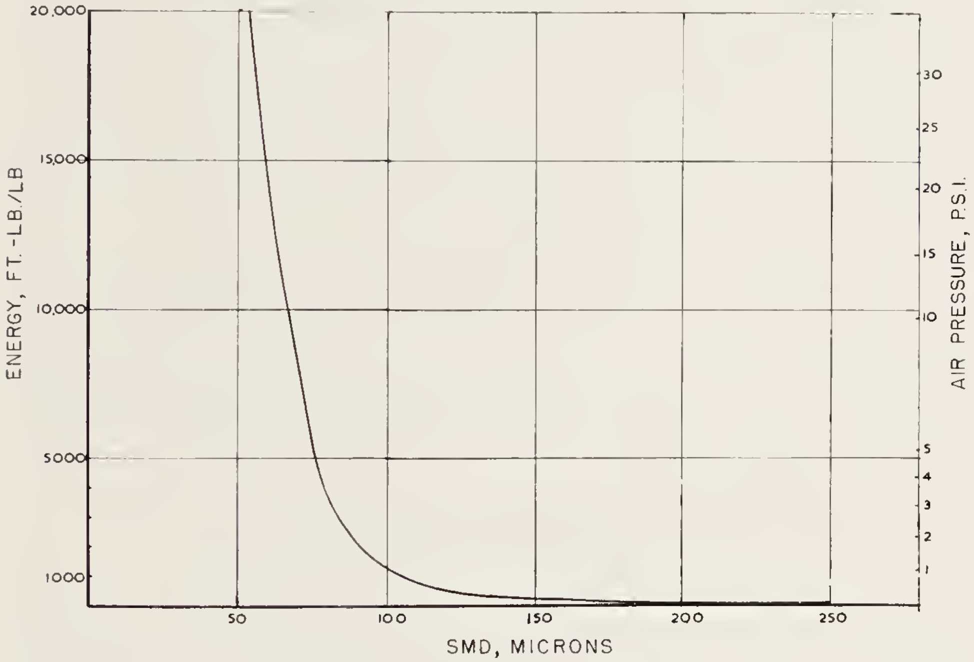


Fig. 16. Drop size energy relation at air/liquid ratio of 1 lb./lb.

High-pressure blast atomizers have so far had little application for spraying. They are commonly employed with liquid fuels and in paint sprayers where a high jet momentum and narrow angle of spray is required. The medium- and high-pressure types yield very small drops and a wide drop spectrum, and the mean drop size depends markedly upon the air/liquid/weight ratio (see Section V.C.3).

Energy requirements for twin-fluid atomizers. It is commonly supposed that blast atomization requires a much greater energy consumption than atomization by hydraulic pressure. With proper prefilming of the liquid before the air accelerates it, however, the amount of energy required is reduced.

The rate at which the energy has to be increased to reduce the drop size below 100 μ can be seen in Fig. 16. This plot gives the kinetic energy in a simple unidirectional air jet expanding isentropically from an orifice, such as a simple injection atomizer giving a flow pattern as represented in Figs. 27 and 29. It is observed that a mean drop size of 110 μ at an air/liquid ratio of 1 lb./lb. can be obtained with an expenditure of only 1000 ft.-lb., but to achieve a drop size of 50 μ requires well over 20,000 ft.-lb. At low velocities of a few inches water gauge the expansion of the gas may be partially isothermal so that the energy in the jet would be higher.

It is common practice with commercial atomizers to operate between about 1 and 10 p.s.i. and for the smallest drop size to use pressures between 15 and 20 p.s.i. to obtain sonic velocity. Practically no atomizers have, so far, been designed to effectively employ velocities of Mach 1–3 or more, corresponding to the proper isentropic expansion of a gas at high pressure.

III. Principles of Disintegration

An atomizing device to give control and adjustable drop size within narrow limits is universally required. The achievement of this can only be brought about by a close study of the mechanism of breakup of the liquid sheet and thread.

A. PHOTOGRAPHIC METHODS OF RESEARCH

Little can be discovered of the mechanism of disintegration without the aid of high-speed photography. Many experimenters have employed photography. Scheubel (87) and Lee (59–62) showed in spark photographs the ligament formation by the action of the air tearing the mass of liquid apart. Fogler and Kleinschmidt (17) showed that pronounced waves may be superimposed upon a conical sheet. Fraser *et al.* (23) found that the spraying of an emulsion of oil in water results in a network of unstable liquid threads arising from perforations in the sheet. Tanasawa (97) applied photography to twin-fluid atomizers.

Simons and Goffe (91) also showed that disintegration can occur through the superimposition of a wave motion on the sheet. Recent investigations have shown that the interpretation of the mechanism is complex and requires the utmost in photographic technique (12,14, 16,22,28,30,56,71,112).

A simple sheet of liquid issuing from a spray nozzle at a pressure of 50–100 p.s.i. will eventually disintegrate into drops, the size range of which will be usually between 5 and 300 μ in diameter. The choice of optical system and sensitized material for photographing such spray particles is not simple, for the following reasons:

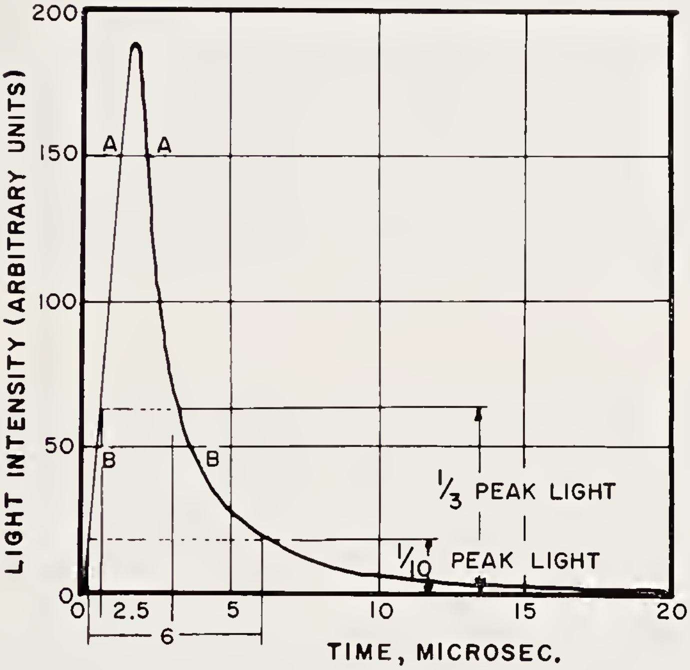


Fig. 17. Variation of effective exposure time with photographic light output

(*1*) The breakup of small filaments is so rapid, about 10^{-6} seconds for a filament of radius 5 μ, that it is only recorded with difficulty.

(*2*) The value of the smallest particle diameter resolved is dependent upon both the liquid velocity and the possible exposure time. For example, at only 50 p.s.i. with water the distance traveled in one microsecond by a particle is 26 μ, and at 100 p.s.i., 37 μ. If we accept the criterion that no particles need be resolved which will travel more than their own diameter during the time of the light flash, then these figures are the smallest diameter particles resolvable with a 1.0 microsecond exposure.

(*3*) The degree of resolution should be maintained over sufficient depth of field to cover the area of the spray under examination. The depth of field required depends upon the geometry of the spray. This can be smaller for flat sprays than for conical sprays. The requirements of depth of field and high resolution are mutually conflicting.

(*4*) The intensity of a light flash from a discharge tube rises to a sharp peak followed by a relatively longer decay period. The effective photographic exposure, therefore, may vary within wide limits depending upon the lens aperture and the sensitivity and the gamma of the emulsion. Figure 17 shows how the effective photographic duration of the flash depends upon the level of light intensity utilized. At high levels of light intensity (AA) the flash can be of relatively short duration, whereas at lower light intensity (BB) it may be four or five times longer. The figure shows that at one-third peak light the duration is 2.5 microseconds whereas at one-tenth peak light it is 6 microseconds. About 0.1 microsecond is the practicable minimum by this method.

(*5*) The theoretical resolving power of a particular lens can be calculated for a particular depth of field, taking account of lens aberrations.

(*6*) The resolving power of an emulsion will vary with different degrees of exposure and of contrast in the object as well as with processing conditions.

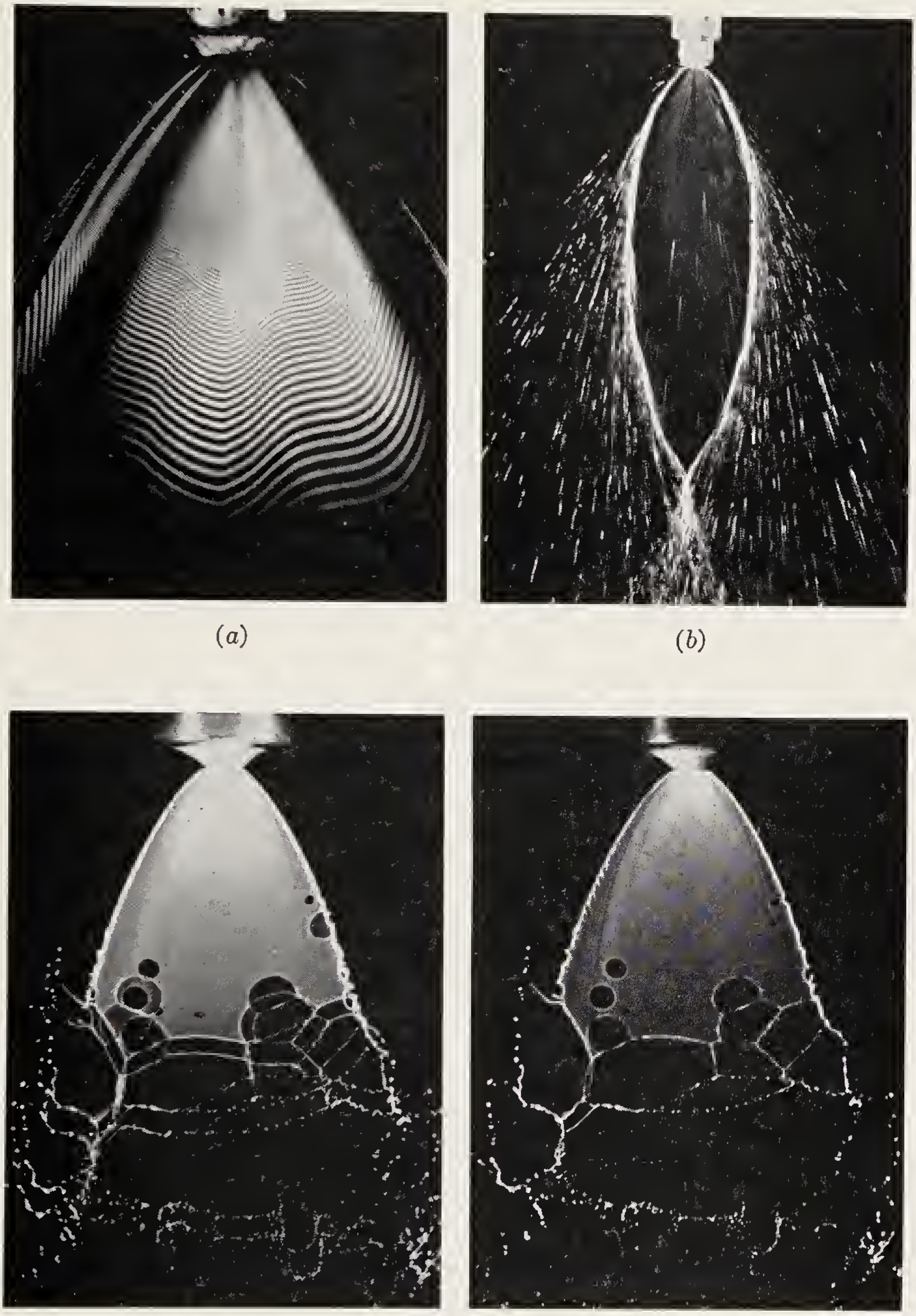
To enable a choice of lens to be made, the following relation can be derived for the magnification at which the resolution with an emulsion of given resolving power is a maximum over a given depth of field with an ideal lens:

$$m_{\text{opt.}} = (2\,cw/d_f)^{1/2} \tag{10}$$

At the optimum magnification the diameter of the smallest particle which should be resolved is given by

$$D_R = (wd_f/2\,c)^{1/2} \tag{11}$$

where m is the magnification; c is the effective lens aperture; w is the diameter of smallest circular image which the emulsion is capable of resolving; d_f is the depth of field; and D_R is the diameter of smallest particles which the optical system can resolve.



(a) (b)

(c) (d)

Fig. 18. Photographic techniques, three exposures: (a) centisecond (light interference), (b) millisecond (point light reflectors), (c,d) microsecond (diffuse reflection, discriminating light quality).

The resolution depicted by this equation is independent of the focal length, but a long focus is desirable.

In applying photographic technique it has been found particularly advantageous to study laminar sheets for the above reasons, and several advances in method have been made (10,21,24,27). For the study of liquid sheet disintegration a variety of information is required and a number of techniques have to be employed (11,25), for although one photograph taken at the required speed level may give the information looked for, it does not necessarily disclose the whole truth. Three different techniques are illustrated in Fig. 18. Figure 18*a* is a centisecond exposure using an interferometric method of lighting to enable the thickness of the sheet to be determined and its variation as the sheet develops from the nozzle; Fig. 18*b* shows the direction of flow of liquid within the sheet and also as it passes through the rim into the atmosphere. The photograph is a millisecond exposure using light reflected from tracer particles of metal (aluminum) within the liquid (water) which act as point reflectors. Figure 18*c* shows a double microsecond flash exposure using a known time delay between exposures to enable the drop movement and velocity to be determined and Fig. 18*d* is a simultaneous reference exposure of the first position, to aid in the analysis of the double exposure in (*c*).

B. THE MECHANISM OF DISINTEGRATION

The fundamental principle of the disintegration of a liquid consists in increasing the surface area of a sheet or thread until it becomes unstable and disintegrates.

The disruption of the liquid as it is discharged from a nozzle is affected by: (*1*) disturbances in the fluid flow within the atomizer, which cause turbulence in the liquid—turbulence varies with liquid velocity, viscosity, and density and dimensions of spraying device; (*2*) the properties of the gaseous medium into which the stream is discharged: namely, density and viscosity; and (*3*) the physical properties of the sprayed liquid: namely, viscosity, density, and surface tension.

In any atomizer the kinetic energy originally imparted to either the liquid or the gas is shared with the other phase, and the kinetic energy of the combined phases is dissipated in turbulent flow. The surface energy, defined as the liquid surface area times the surface tension created in the process is only a very small fraction, about $^1/_{1000}$ part, of the total energy originally available. In the pressure atomizer the greatest portion of the kinetic energy is retained by the spray drops so that they penetrate into and mix with the atmosphere into which the spray is ejected. In the twin-fluid type the excess energy in the gas is not necessarily wasted because it may be employed in an injector system to entrain more atmospheric air, as is commonly done in combustion equipment.

The classical example of drop formation is the breakup of a liquid thread or column which becomes unstable under surface tension alone. Rayleigh (83) showed that the most probable disturbance for a nonviscous liquid jet *in vacuo* is an expansion and contraction which occurs over $4^1/_2$ diameters length of the column. Swellings on the column with wavelengths equal to about this figure grow the fastest and represent the most probable breakup length. Drops resulting from this most rapidly creating disturbance will have a diameter equal to 1.89 times the diameter of the undisturbed jet. The surface area of the drops per unit liquid volume will be 80% of the surface area of the column. Thus there is a 20% decrease in suiface area during this breakup process.

Tyler (103) determined experimentally the mathematical predictions of Rayleigh and found an average figure of $4.69d$ for the maximum wavelength instability. The fundamental concepts of jet instability breakup apply qualitatively to all types of atomizers. The Rayleigh theory indicates that the lifetime of a ligament forming small drops is exceedingly short, the time constant of the amplitude for the most probable wavelength is

$$\lambda = 0.34\ (8\ \gamma/\rho_L D^3)^{1/2} \tag{12}$$

where D is the diameter of the liquid filament; ρ_L is the liquid density; and γ is the surface tension. The formula shows that for a filament of radius 5 μ, the breakup time or lifetime is of the order of 10^{-5} sec.

Weber (110) made a more general mathematical analysis of a jet having viscosity, and showed that the wavelength/diameter ratio required for maximum instability of a viscous jet is

$$\lambda/D_0 = (\pi/2)(1 - 3\ \nu/\sqrt{\rho_L \gamma D_0})^{1/2} \tag{13}$$

In this equation, when ν, the kinematic viscosity, is zero, the wavelength found is 4.44, nearly the value given by Rayleigh.

In liquid jets where the principal velocity component is in the longitudinal direction, the breakup distance decreases with increase of pressure and increases with increase of viscosity. If the jet is in laminar flow, some external disturbance is essential for its disintegration, apart from Rayleigh breakup; but if the jet is turbulent throughout, it will break up without the application of any external force. Upon leaving the nozzle radial velocity components within the jets are no longer confined by the orifice walls; thus disintegration will occur as the surface tension is overcome. The jet will be influenced to break up by any factor affecting the general turbulence state of the liquid as it leaves the orifice, such as uneven roughness of approach to the orifice or damage to the leaving edge. Regardless of all other effects the disintegration will always be favored by air friction, which increases with air density.

Because of surface tension the configuration of a large surface area is unstable and on undergoing random disturbances, some of which are more probable and speedier than others, it breaks up into a drop system of smaller surface area. The difference in area between the surface of the sheet and the surface of the drops formed therefrom is shown in Table V. In a small fan-shaped sheet the reduction of area when the drops are formed can be as much as six times. With a conical sheet from a swirl nozzle the difference is not so great at low pressures, and in fact, the difference is reversed at high pressures where the drops have the larger area caused by further disintegration due to air friction after leaving the sheet.

TABLE V
A Comparison of the Surface/Volume Relationship
of Drop and Spray Sheet

	Fan nozzle		Swirl nozzle			
Flow number (FN)	0.74		0.20			
Pressure (P), p.s.i.	25	100	25	50	100	200
Surface mean diameter (SMD) of water drop	170	107	129	95	80	71
Surface volume ratio, $cm.^2/cm.^3$						
Of sheet	1850	1850	760	700	440	460
Of drops	350	560	460	630	750	850

The formation of ligaments or fine threads from the sheet is a necessary stage before the production of drops, and these can be formed directly from a free edge or in any boundary. A leading edge is formed when equilibrium exists between surface tension and inertia forces.

To open a sheet against the contracting force of surface tension, a minimum sheet velocity is required. As previously mentioned (Section II), this velocity can be produced by pressure on the liquid, by centrifugal force, or by aerodynamic action. Increasing the velocity expands and lengthens the sheet to an optimum distance; then, with further increase, the sheet contracts toward the orifice by disintegrating earlier, owing to increased turbulence and air friction.

In Fig. 18*b* it is seen that the stream lines in a fan sheet are straight and radial and unaffected by the curved boundary and, also, that the drops leave the edges tangentially at an angle different from that of the stream lines. Many of the drops at the base of the sheet cross over from one side to the other. If the liquid had no surface tension, a fan sheet would take the form of a sector of a circle. Surface tension forces bend the rim inward; and, since the shape is determined by the balance of inward surface tension force opposed to outward rate of change of momentum, the bending inward of the rims is most apparent at low ejection pressure. From photographs such as Fig. 18*c,d* it is found that the velocity is constant throughout the sheet and that there is no change in velocity between the drops leaving it either at the leading edge or from the sides. This indicates there is no measurable energy loss during the breaking off of ligaments and drops.

With a relatively nonviscous liquid the thickness of the sheet at point of rupture and the velocity of rupture are related by

$$u = (4\gamma/\rho_L h)^{1/2} \tag{14}$$

where h is the thickness of the sheet, and u is the velocity at rupture. The thickness of the sheet can be determined by the interferometric method of Fig. 18*a*, where the variation of thickness between the fringes is 0.2 μ and the sheet thickness is 7.5 μ at 2 cm. from the nozzle.

Some characteristic measurements of a fan spray sheet from a nozzle of the kind in Fig. 2*b* using water are given in Table VI.

Four modes of disintegration in a sheet have been defined: "rim," "wavy sheet," "perforated sheet," and "air impact" (28), although there is only one mechanism of disintegration, namely, the breakup of the liquid threads.

Because of surface tension any liquid boundary is continually con-

TABLE VI
Properties of Spray from Single Hole Fan Spray Atomizer, with Water

	Nozzle size				
	1	2	3	4	5
Flow number (FN)	0.35	0.74	1.15	1.62	2.17
Discharge coefficient (C_Q)	0.800	0.878	0.867	0.943	0.953
Velocity of sheet at 100 p.s.i., cm./sec.	2980	3270	3230	3520	3540
Thickness at breakup, μ	3.2	6.6	10.9	14.0	19.5
Surface mean diameter (SMD), μ	84	107	125	143	173

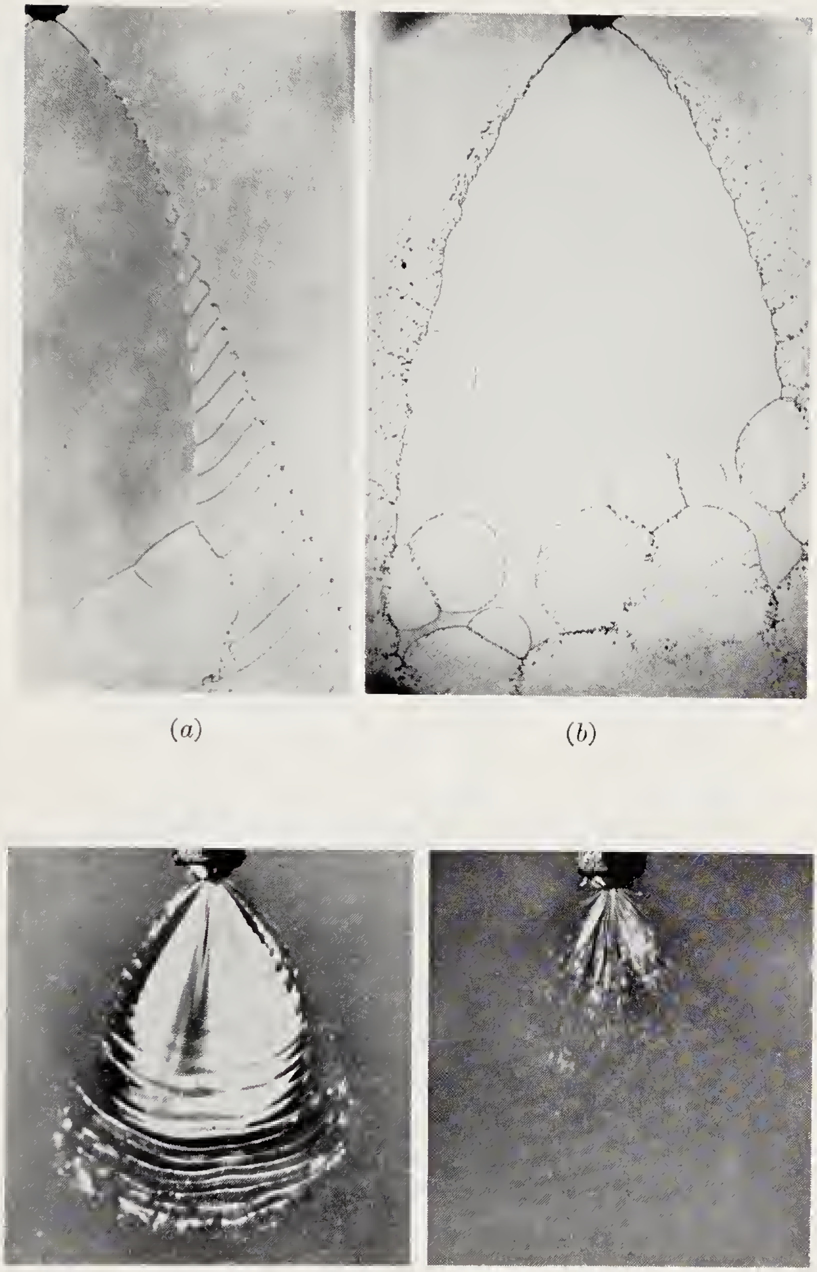
tracting, and this phenomenon produces "rim" disintegration (Fig. 19*a*). Threads are pulled out from the rim during contraction and these produce larger drops than those formed from the leading edge of the sheet. This mode is most prominent under conditions of low velocity, high surface tension, and high viscosity. The photograph is of 65% glycerine/water sprayed *in vacuo* (29.25 in. Hg) at a pressure difference of 25 p.s.i.

In the second mode—"wavy sheet" disintegration (Fig. 19*c*)—the sheet stability is such that major wave disturbances caused by the atmosphere disintegrate it by tearing whole surfaces away before the leading edge is reached.

Some workers stress the importance of air friction, while others believe turbulence in the nozzle to be the most influential factor in this form of disintegration. Evidence with flat sheets points to the conclusion that they are more stable than simple liquid jets at high velocity and that liquid turbulence plays the minor role.

It is observed that an atomizer with a large orifice spraying into the atmosphere produces a turbulent sheet at a pressure at which a nozzle with a small orifice would produce a placid one. Most of the sheets of liquid produced from nozzles in practical applications have a high velocity and turbulent flow conditions exist in the orifice. This turbulence on the sheet is superimposed on any aerodynamic wave motion, and it may give rise to higher friction and greater entrainment of the atmosphere.

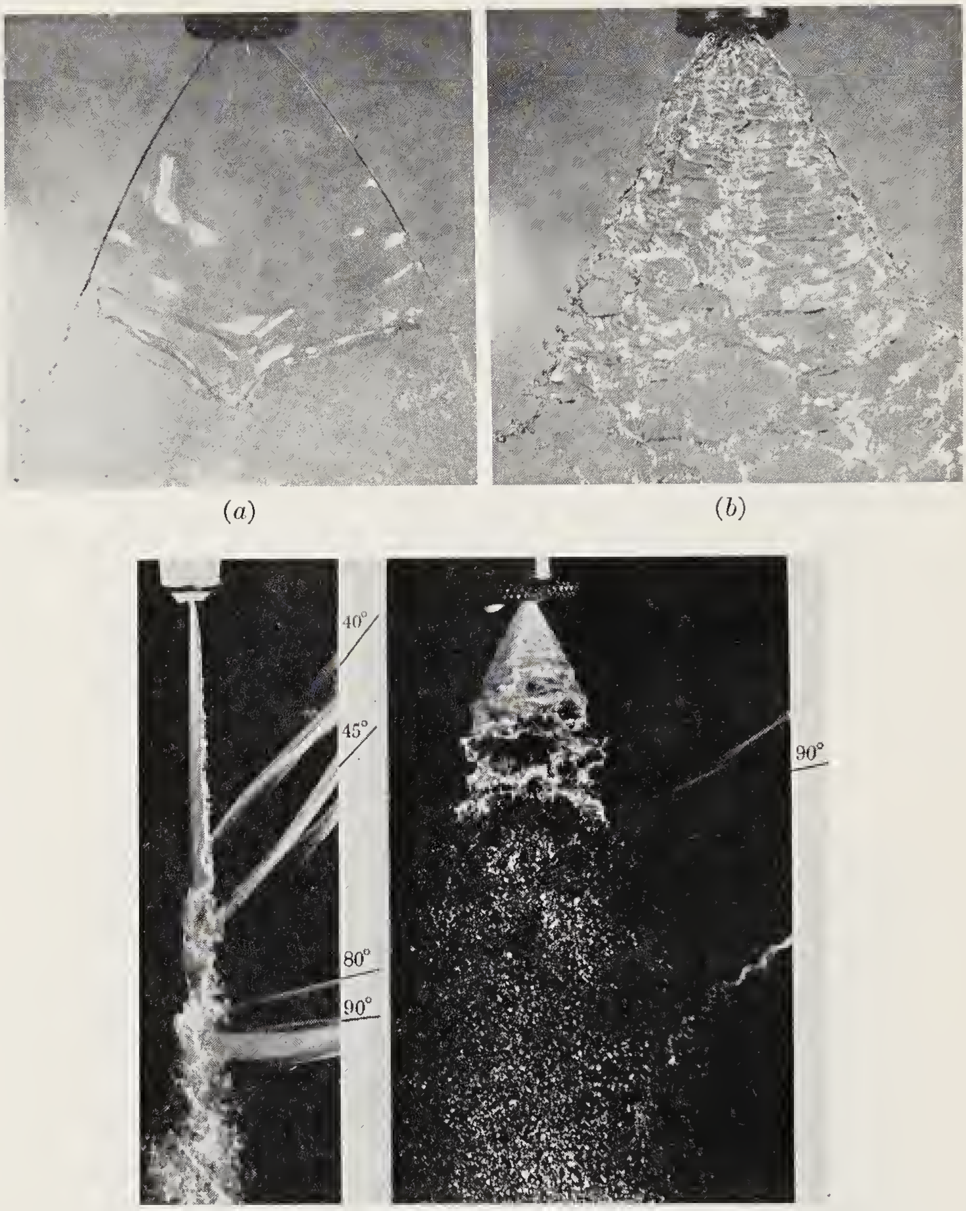
Figure 20 shows two photographs of fan nozzles working at Reynolds numbers of 15,000 and 41,000, respectively, the liquids having equal velocities of 48 ft./sec. Figure 20*a* shows a relatively placid sheet with the characteristic aerodynamic waves near the leading



(*a*) (*b*)

(*c*) (*d*)

Fig. 19. Modes of disintegration: (*a*) "rim" disintegration, glycerine and water, 15 centipoise viscosity, velocity of sheet 58 ft./sec. (*in vacuo*), (*b*) "perforated sheet" disintegration, water, velocity 85 ft./sec. (*in vacuo*), (*c*) "wavy sheet" disintegration, water, velocity 120 ft./sec. (in atmosphere), (*d*) "air impact" disintegration, water, 350 ft./sec. (in atmosphere).


(a) (b)

(c) (d)

Fig. 20. Influence of Reynolds number on sheet disintegration: (*a*) Re 15,000, (*b*) Re 41,000. Entrainment of air by the sheet: (*c*) fan-shaped sheet, edge on, (*d*) conical sheet.

edge. Figure 20*b* shows the other type of instability induced by turbulence in the nozzle, which produces irregular waviness covering the whole area of the sheet.

When spraying into the atmosphere it is impossible to separate these two effects. *In vacuo* air reaction can be eliminated and turbulence can be induced by increasing the working pressure. The examination of a large number of photographs of sheets *in vacuo* gives the limits and ranges of Reynolds number for laminar, transition, and turbulent flow. Table VII shows that the critical Reynolds number at which turbulence sets in is between 18,000 and 37,000, and this indicates that the fan spray sheet is more stable than the simple jet, which has a critical Reynolds number of 2300. The turbulence on liquid sheets appears to have very little influence on the disintegration and does not seem to have a noticeable mutual effect with the aerodynamic waves.

TABLE VII
Reynolds Number for Liquid Sheets

	Nozzle orifice area, cm.^{2} × 10^{-3}		
	1.71	2.89	5.60
Laminar			
Pressure	0–250	0–110	0–12.5
Reynolds no.	0–23,200	0–19,600	0–9200
Transition			
Pressure	300–450	115–200	15–25
Reynolds no.	25,400–31,100	21,400–26,900	10,000–13,000
Turbulent			
Pressure	600——	400——	50——
Reynolds no.	35,900——	37,300——	18,400——
Mean film thickness at breakup, μ	1.4	1.9	3.1

Aerodynamic waves arise from an interplay between aerodynamic forces and surface tension. A small protuberance on the sheet is subject to two opposing forces. Surface tension tends to flatten out the disturbed surface, whereas the air flowing relative to the sheet experiences a local decrease in pressure corresponding to its increased velocity and this tends to draw out the liquid from the plane of the sheet. When aerodynamic forces overcome the surface tension forces, any small disturbance present in the sheet grows rapidly into major waves, the rate of growth of which is exponential. The wavelength of the most unstable wave having the maximum amplitude growth rate is given by

$$\text{Wavelength} = 4\pi\gamma/\rho_a U_0^2 \tag{15}$$

where γ is the interfacial tension, ρ_a is the air density, and U_0 is the liquid velocity relative to the air. Thus the higher the velocity (pressure) and the lower the surface tension, the shorter the wavelength and the more vigorous the breakup (94).

When the air density is reduced, the wavelength increases progressively while the sheet enlarges further. A point is ultimately reached where the waves disappear completely and the sheet is perfectly flat. Under these conditions, perforations appear on the very thin sheet, which expand rapidly into a network of ligaments. This is "perforated" disintegration.

In "perforated sheet" disintegration (Fig. 19*b*) the leading edge disintegrates to a network of threads. Point disturbances on the sheet (12) puncture it when they have reached a thin enough region, and the resulting holes expand regularly by surface tension so that they remain approximately circular until they coalesce, forming long threads which disintegrate when they become unstable. Threads which escape strong air reaction and which are formed in an orderly manner disintegrate uniformly according to the Rayleigh theory.

The rate of growth and cause of perforations on liquid sheets has been studied *in vacuo* over a wide range of injection pressures, and it has been shown that the rate of expansion of a perforation is independent of its radius. An equation for the rate of growth of a perforation was developed to give an expression for the location of the origin of the observed hole. It was shown that the average hole originates in the central region of the sheet and that the radial line passing from the center of the perforation is usually less than 1 cm. to the orifice. The expanding perforations travel from their origin only short distances of about 0.5–2.5 cm. before they coalesce and cause disintegration, and the average lifetime of the hole is approximately 1 millisecond. A significant number of perforations appear in the upper part of the sheet at higher pressures.

Although there are a number of known causes for perforations in the sheet (see Section III.D), the reason for perforations in pure liquids *in vacuo* is more difficult to explain. One reason suggested (26) is that these random disturbances have their origin inside the nozzle and are due to turbulence above a particular Reynolds number. Another reason suggested is the bombardment of the sheet *in vacuo* by extraneous liquid particles (43). Figure 5*d* shows a sheet of distilled water at 25 p.s.i. differential pressure in an air density of 0.05 g./liter, 28.75 in. Hg vacuum.

Clearly the orderliness of the manner of disintegration of the sheet and the uniformity of production and diameter of threads have a great influence on the drop size distribution. "Rim" disintegration gives large drops together with a number of small satellite drops, as are also obtained from a spinning disk at a low flow rate (see Fig. 39*a*,*b*). "Wavy sheet" and "air impact" disintegrations are highly irregular, and thus the drop sizes are very varied. Perforations occurring in the sheet at the same distance from the orifice have a similar life; thus the threads can be uniform, producing uniform drops. In "perforated sheet" disintegration, therefore, the drop size is partly con-

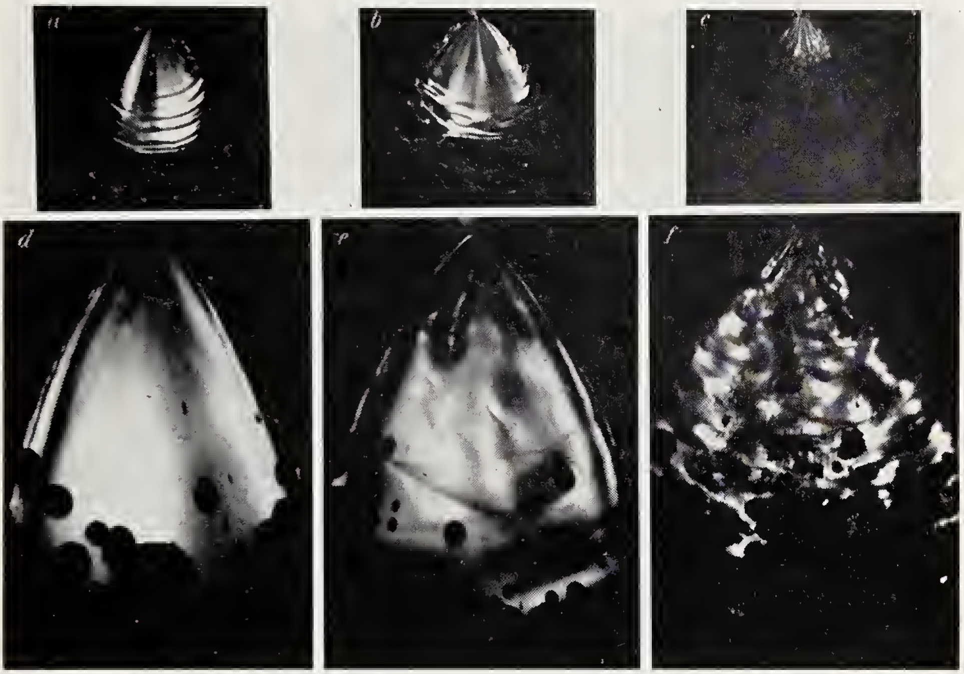


Fig. 21. The effect of differential injection pressures and atmospheric density on turbulence in liquid sheets: (*a*) 100 p.s.i., Re 18,600, atmospheric density; (*b*) 122 p.s.i., Re 20,600, atmospheric density; (*c*) 740 p.s.i., Re 50,700, atmospheric density; (*d*) 100 p.s.i., Re 18,600, sub-atmospheric density; (*e*) 125 p.s.i., Re 20,900, sub-atmospheric density; (*f*) 800 p.s.i., Re 52,700, subatmospheric density.

trolled by the number of ruptures (see Figs. 4*b* and 19*b*), and the drop size will bear a relation to the number of ruptures per unit area and the thickness of the sheet (81,95).

$$d = 1.89\,(8h^2/\pi^2\sqrt{3n})^{1/4} \tag{16}$$

where n is the number of rupture points per unit area, and h is the thickness of the sheet. Unfortunately the irregular areas of liquid which occur when a number of holes expand toward each other and the rims coalesce produce a much wider variety of drop sizes.

Figure 21 illustrates some of these effects. It shows a comparison between water sheets in the atmosphere and in vacuum at equal pressure differences using the same nozzle. In vacuum the sheets are much larger and more placid at any injection velocity, but at high velocity (Reynolds number >20,000) the sheet is decidedly turbulent and irregular cavities and ridges occur quite unlike those caused by air reaction (compare Figs. 21*c* and 21*f*). In a vacuum, where the atomizing influence of aerodynamic waves is absent, the drop size is larger, although the thickness of the sheet is less than in atmosphere. In the atmosphere the waves increase the frictional drag of the air and the surrounding air is entrained by the sheet. This is illustrated in Fig. 20*c*, where the motion of the air is made visible by smoke trails. Where the wave motion is most pronounced air flows into the sheet in a perpendicular direction, whereas near the orifice where the sheet is more placid the air entrainment is at an oblique angle. With conical sheets the air entrainment has an additional effect. The pressure inside the conical sheet near the nozzle is reduced by continual entrainment and, as a result, the surrounding air moves inward toward the axis in a direction normal to the surface of the cone. This additional air motion sets up disturbances which enhance the wave motion (Fig. 17).

At very high injection pressures both the liquid turbulence and the air friction are very great, and the disruption of the liquid in this case is more nearly that of a twin-fluid atomizer, where the velocity difference of the fluids is so high that air reaction plays the major role. Thus a fourth mode of disintegration may be defined, where the shatter of a small but thicker sheet takes place close to the nozzle (Fig. 19*d*) (see Section IV).

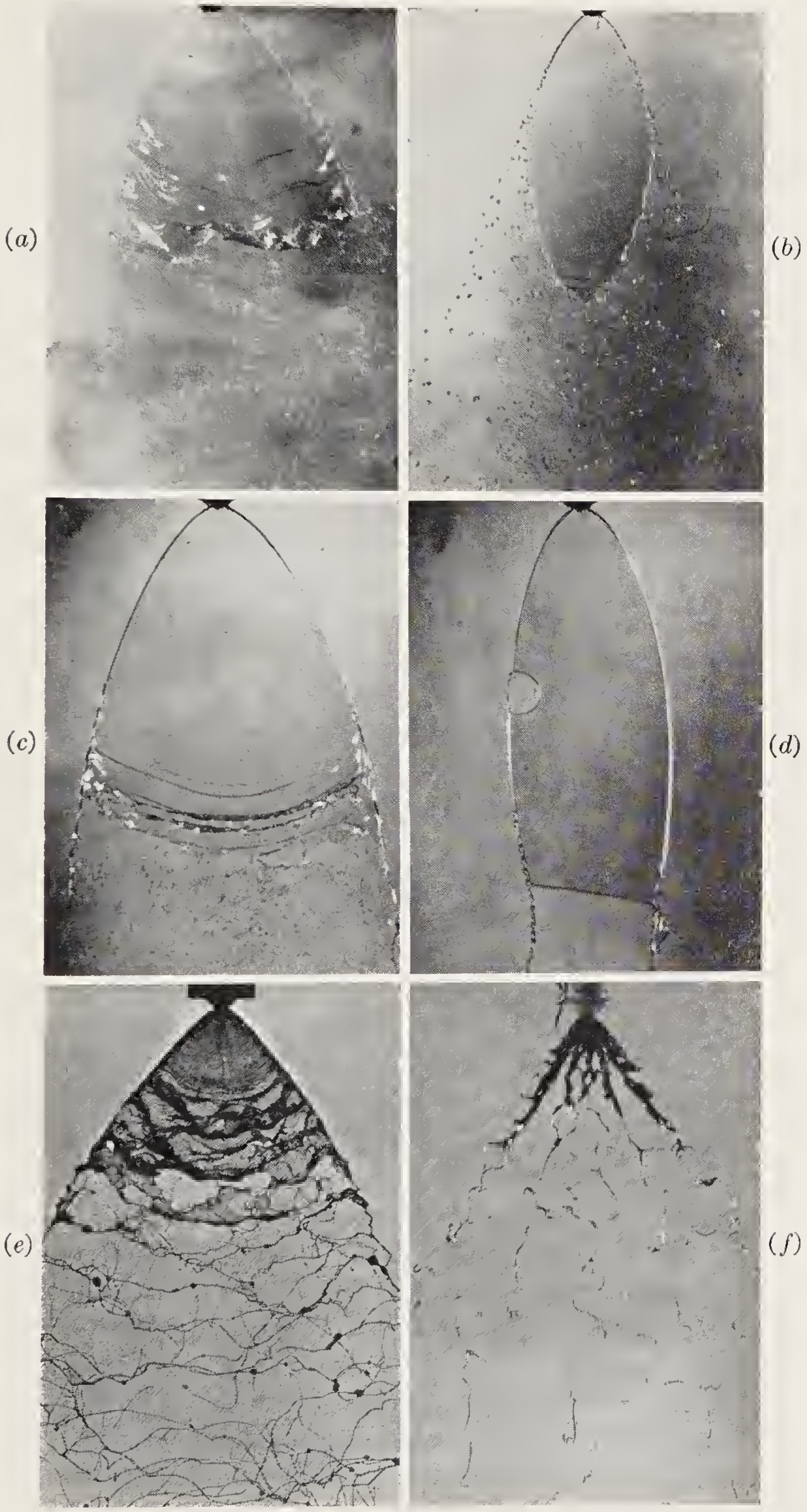


Fig. 22. The combined effects of viscosity and surface tension with approximately constant density: (*a*) ethyl acetate, 0.5 cs., 24 dynes/cm.; (*b*) water, 55°C., 0.5 cs., 68 dynes/cm.; (*c*) butyl alcohol, 5.3 cs., 24 dynes/cm.; (*d*) glycerine/H_2O, 5.3 cs., 68 dynes/cm.; (*e*) petroleum gel, circa 5 g. Gardner; (*f*) sodium, 180°C., 0.6 cs., 187 dynes/cm.

C. EFFECT OF LIQUID PROPERTIES ON DISINTEGRATION

The two liquid properties which have most importance in spraying are the surface tension, which resists the formation of new surface, and viscosity, which also resists the formation of new surface by delaying the internal flow necessary to create the larger surface area. During the final stage of disruption to drops, however, the viscosity opposes the surface tension forming drops and, if the viscosity is great enough, no drops will be formed but only threads. Density has little effect, particularly in the small range commercially encountered in spraying (0.78–1.2 g./cc.). With the exception of liquid metals and fused salts the surface tension of most liquids is below that of water, 73 dynes/cm., so that the commonly encountered range is 20–80 dynes/cm. Viscosity has the greatest range, the limit being the highest viscosity that the atomizer is capable of handling to give the desired drop size. The maximum limit in atomization is about 100 cs.

The combined effect of viscosity and surface tension with an approximately constant density (about 1.0) is illustrated in Fig. 22. The photographs are of fan sheets of different liquids from the same nozzle at the same liquid velocity. They show the marked effect of increasing the viscosity approximately ten times at two levels of surface tension and also the effect of increasing the surface tension approximately three times at two levels of viscosity. Increase in viscosity lengthens the sheet, while increase of surface tension contracts it and makes it more placid.

D. EFFECT OF ADDITIVES ON DISINTEGRATION

To obtain improvement and modification of the biological result of spraying it is quite common for additives to be used with the pesticidal carrier, which is most commonly water or a light fuel oil. These additives, commonly called spreaders, stickers, and emulsifiers, may consist of a vast variety of materials, some soluble and some insoluble. In many cases the pesticidal chemical may be insoluble and require the addition of a wetting agent or emulsifier, such as with fungicides of copper and sulfur. Spreaders or wetting agents are mainly soaps or surface active agents such as organic sulfates and sulfonates and fatty acid derivatives, which have to be selected according to the pH required. Materials referred to as stickers may be many common ingredients such as wheat or soya bean flour, resin-oil mixtures, and even powdered glue.

The object of stickers is to form adherent coatings so as to improve the resistance of the deposit to weather conditions. The addition of aminoplasts and a hardening agent is one way of converting an original soluble addition to an insoluble state. A further method is the addition of a water-insoluble organic acid with adherent properties capable of forming a water-soluble ammonium salt which, on drying, splits up into free insoluble acid and ammonia.

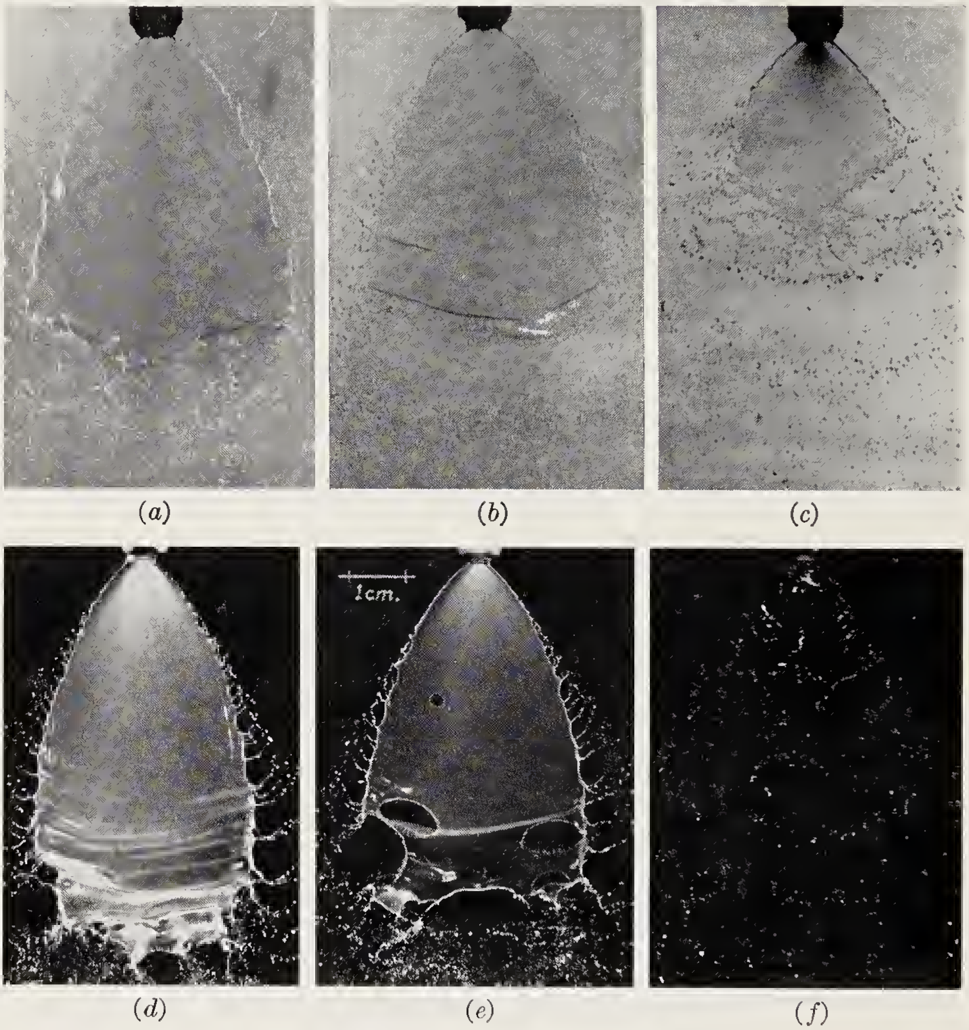
With the exception of the surface active agents it is quite clear that little is known about the effect of such additives on the disintegration of the sprayed liquid and the resultant drop size. Much more research is also required to discover how such additives modify the biological effect of the active ingredients of the spray.

Generally speaking, the agricultural engineer has not been very much concerned with the effect of the physical properties of the liquids sprayed, except as regards the mechanical convenience in handling, such as viscosity and corrosiveness. It is very tempting, therefore, to neglect the variations brought about by change in physical property in the liquid vehicle. So far little attempt has been made to impose a preselected and controlled physical condition on the sprayed liquid vehicle so as to modify the spray by delaying or changing the formation of drops.

1. Effect of Suspensions

Where liquid particles are suspended in the vehicle to form an emulsion, or solid particles are merely added, the stability and mode of disintegration of the sheet are found to depend quite markedly upon the type of suspended particle and whether the particle is wettable or unwettable.

When a wide range of different types of particle, varying in size from 3 to 60 μ in diameter, is introduced into a sheet of water, or water plus wetting agent, or alcohol, it is found that (*1*) low concentrations (0.05% weight volume) have no effect on the process of disintegration even when the particles are larger than the sheet thickness (about 7 μ at 2 cm. from the nozzle); (*2*) where the particles were wetted by the liquid they have no effect on the manner of disintegration irrespective of their size (it appears that the large particles are held by the sheet through the adsorbed films on their surface); and (*3*) when the particle concentration is increased to form a slurry there are marked differences in the manner of breakdown of the sheet. Figure 23 shows the effect of the addition of increasing percentages of fuller's earth to water. Figure 23*b* is of a 5% weight volume addition, which has little effect; Fig. 23*c* is a 20% weight volume suspension, and the sheet is observed to break down very much closer to the orifice through the formation of holes and tears.



(*a*) (*b*) (*c*)

(*d*) (*e*) (*f*)

Fig. 23. The effect of suspensions: (*a*) water, (*b*) water + 5% (wt./vol.) Fuller's earth, (*c*) water + 20% (wt./vol.) Fuller's earth. Perforations produced in a sheet by the addition of an electrolyte to a water-oil emulsion: (*d*) no electrolyte, (*e*) + 0.2% sodium chloride, (*f*) + 0.4% sodium chloride.

This considerable change in the mode of disintegration has an influence on the drop size.

When suspensions of unwettable particles are used, such as sulfur in water or oil in water, they have a marked effect and cause perforations in the sheet. If the oil globules or unwettable solid particles are very small, the sheet will break down through the formation of waves in the usual way. With larger particles, however, a perforated sheet is produced when the sheet has thinned down to approximately the diameter of the largest suspended particle. Thus the larger the particles in suspension, the earlier the sheet will perforate and the greater will be the influence on drop size.

Figure 23*d* is of soluble oil in water and illustrates this phenomenon. When the soluble oil is prepared in water containing no dissolved salt or electrolyte, the emulsion particles are very small. When the solution is prepared in hard water, or water in which traces of electrolyte are present, the oil particles coalesce and become larger and the perforated sheet effect shown in Fig. 23*e* is immediately observed. With further addition of electrolyte, such as sodium chloride, the particles rapidly coalesce into large globules and the sheet becomes perforated almost immediately it leaves the orifice Fig. 23*f*.

2. Effect of Wetting Agents

The effect of wetting agents is somewhat more complex. The surface tension of an aqueous solution of a surface active agent varies with the age of the surface (1). The tension of a freshly formed liquid surface, as measured by dynamic methods, is equal to that of the pure liquid, and a definite time interval has to elapse before it obtains an equilibrium value measured by static methods. Since the time interval associated with the life of the sheet at 40 p.s.i. is of the order of only 2 milliseconds, the surface tension of the greater part of the sheet is probably that of pure liquid. It has been found, however, that whereas the addition of a wetting agent to pure water gives no observable change in the disintegration, as would be expected for the above reason, when the agent is added to hard water and extremely fine precipitate formed by the chemical reaction between the wetting agent and the dissolved salts in the water behaves in the same manner as a suspension of unwettable particles.

For example, no effect is observed with a wetting agent in distilled water or in the hard water solution of sodium hexametaphosphate. A wetting agent in hard water alone, however, causes the sheet to perforate to an extent depending upon the wetting agent concentration.

Figure 24 shows three photographs of a swirl spray nozzle spraying water at 15 p.s.i.: hard water alone (Fig. 24*a*), and hard water plus calsolene oil, 0.05% (Fig. 24*b*) and 0.25% (Fig. 24*c*). The progressive change and perforation of the sheet are evident. In (*a*) the sheet is unperforated and disintegration occurs from the leading edge only. The contraction of the conical sheet due to surface tension can be observed. In (*b*) the sheet is perforated and disruption and disintegration occur earlier from the threads formed between the expanding holes. In (*c*) disintegration occurs much closer to the nozzle from holes formed almost spontaneously at the orifice. The angle of spray is increased because surface tension has not contracted the film, as in (*a*).

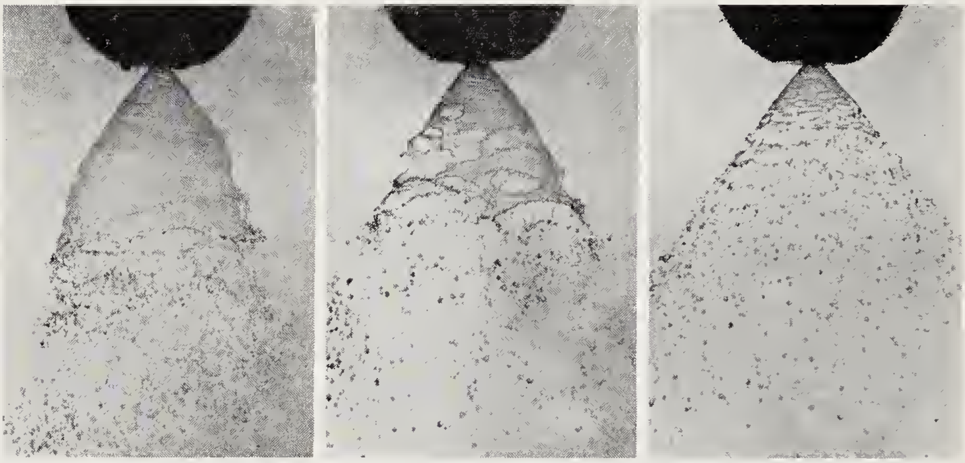

(*a*) (*b*) (*c*)

Fig. 24. The effect of wetting agents in hard water on a conical spray: (*a*) hard water, (*b*) water + 0.05% wetting agent, (*c*) water + 0.25% wetting agent.

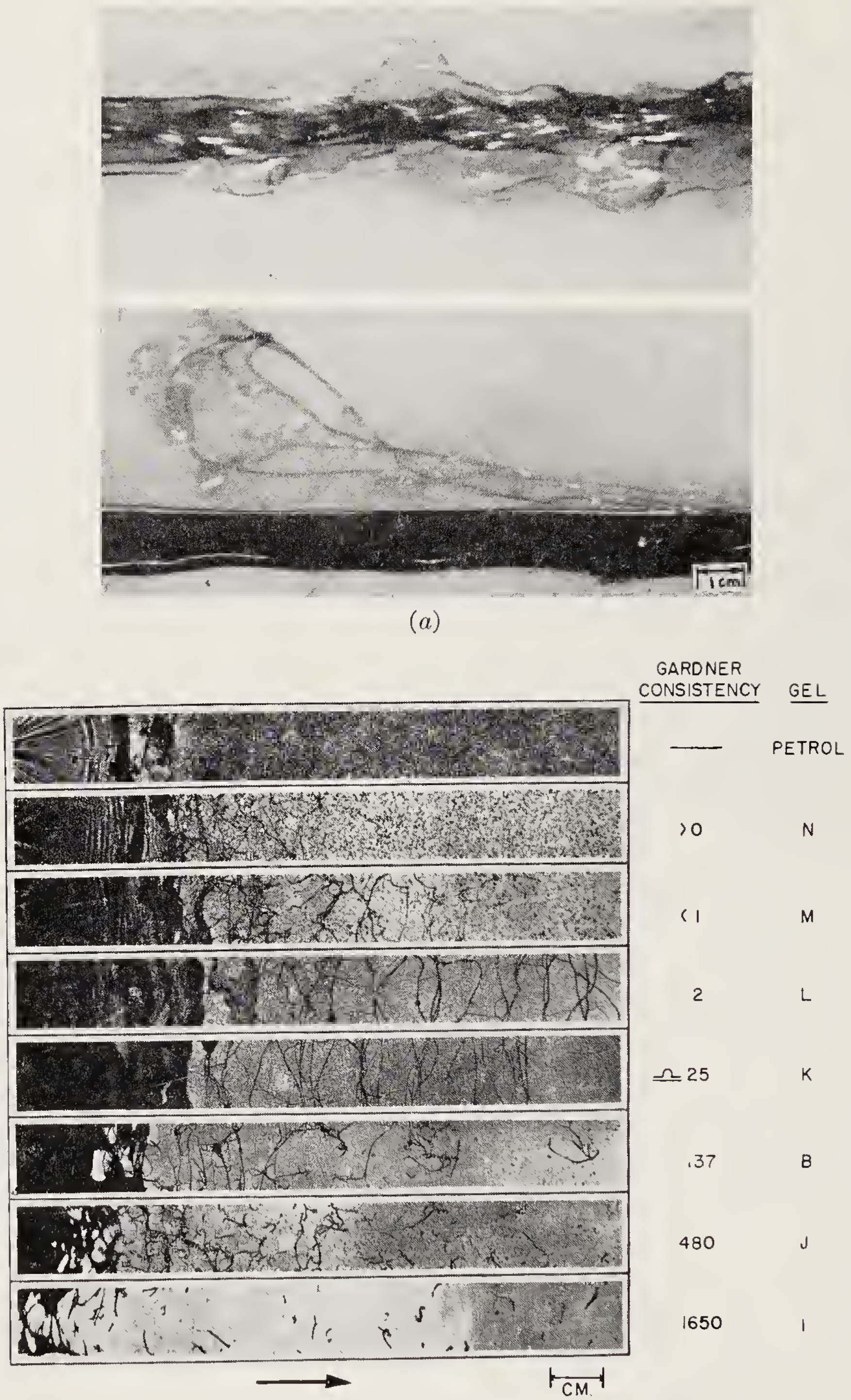
3. *Effect of Visco-elasticity*

The serious ineffectiveness of spraying small volumes of highly concentrated liquid insecticides or growth-controlling hormones, due to drift, is only too evident (see Section VI; Fig. 53). One way of improving the effectiveness would be to prevent the formation of small satellite drops or reduce the number formed, during the process of disintegration of the thread. This can be accomplished by modifying the viscous properties of the liquid vehicle so as to delay or prevent the formation of small drops without change in the atomizer or substantial additions to the liquid.

Generally speaking, the viscous flow of liquids follows Newton's law; that is, the rate of flow and the rate of shear are proportional to the force or pressure applied. The existence of certain non-Newtonian liquids has been known for some years, where the liquid is given a changed viscosity and elasticity, and where the viscosity varies with the rate of shear (34), so that only under high shear conditions is the viscosity reduced sufficiently to allow disintegration to occur. The liquids to which such non-Newtonian properties have been imparted are sometimes referred to as visco-elastic gels, in which cases the non-Newtonian condition may be so pronounced that the liquid appears to be a semisolid gel, but its characteristic is that upon movement or the application of shear stresses it immediately assumes fluidity, reassuming its original semisolid state when stationary.

Visco-elastic properties can be imparted to hydrocarbon or aqueous vehicles by the addition of metallic soaps. In high concentrations these soaps exist in a micellar state, and the change from Newtonian and non-Newtonian viscosity is thought to be due to the formation of interlinked micelles.

Although the measured viscosity of such a gel may be very high, it is found that the surface of a moving jet of gel rapidly forms thin sheets around it which eventually break up into threads (Fig. 25*a*). It has been shown (31) that thin sheets of visco-elastic fuels (gasoline) break up in a different manner according to the amount of visco-elasticity imposed. Figure 22*e* shows a typical fan spray sheet of petrol gel disintegrating into threads with no drops being formed. Figure 25*b* shows the progressive change in disintegration from the same nozzle at the same velocity (66 ft./sec.) with increasing Gardner consistency from 0 to 1650 g. Gardner. These photographs show that at insignificantly low consistencies, as recorded by the Gardner mobilometer, a great change occurs in the manner of disintegration. At about 2 Gardner, threads are formed which are resistant to further breakup and beads are formed on them. Above this consistency, 25–100 Gardner, the threads remain unbroken, and at con-

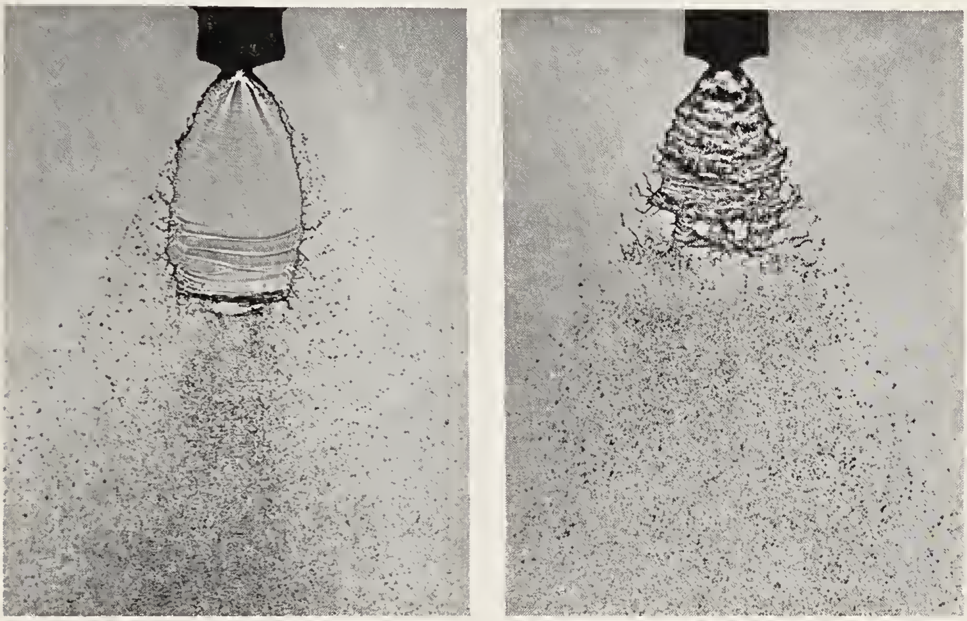

1cm
GARDNER CONSISTENCY
GEL
——
PETROL
>0
N
<1
M
2
L
≏25
K
137
B
480
J
1650
I
CM.

(a)

(b)

Fig. 25. (a) Two instants in the boundary condition of a rapidly moving jet of gel. (b) Mechanism of disintegration of visco-elastic gels.

sistencies greater than 100 Gardner the threads break up into shreds. Control of drop size by preventing the formation of small drops appears to be possible by inducing in the liquid vehicle only a small degree of visco-elasticity. Figure 26 shows the disintegration of a sheet of plain water at 15 p.s.i. and water having a small degree of viscoelasticity induced by the addition of 1% or less of a gel-forming substance (20). The marked reduction in the number of small drops is clearly seen.



(*a*) (*b*)

Fig. 26. Comparison of the disintegration of (*a*) water and (*b*) an aqueous gel from a fan spray nozzle.

IV. Projection and Disintegration of Drops in a Gaseous Stream

In agriculture the ambient spraying conditions are of great importance to achieve the desired deposition. The use of air flow machines, which employ air not only as the atomizing medium but also as the diluting and conveying medium, has brought about a reappraisal of the functions of the agricultural atomizer, and more attention is being paid to the use of the twin-fluid type.

To obtain a uniform dispersion of drops within large-diameter low-velocity air streams from mist blowers, it has been customary to arrange a number of liquid delivery points uniformly disposed within the air stream. The liquid may be delivered from a number of small tubes, or from the trailing edges of a number of aerofoils, without much attempt at atomization, or from a number of small-pressure nozzles, or small medium-pressure twin-fluid atomizers of simple injection design (49). Dispersion of the liquid from a large number of small delivery points is a disadvantage which should be overcome by the use of the larger sizes of medium-pressure twin-fluid atomizers designed to give wide spray angles up to 180°. Other methods which have been employed are to spread the liquid on a screen of rods, or on a rotating blade, spinning disk, or cup. All these methods of spreading the liquid and dispersing it in a low-velocity air stream (100–400 ft./sec.) constitute large low-pressure twin-fluid atomizers.

Three of the characteristic features of the twin-fluid type of atomizer are (*1*) the energy supplied in the gaseous fluid is independent of the liquid flow so that much greater energy may be made available per pound of liquid; (*2*) very wide spray angles can be achieved; and (*3*) small drop size below 20 μ can be obtained at large flow rates.

A need expressed by agriculturalists has been for a device giving no appreciable change in drop size with change of liquid pressure or throughput. The solution to this need is likely to prove impossible with pressure atomizers because in this case drop size is a function of both pressure and flow number (FN) (see Section V.C.1). The twin-fluid atomizer, however, can give nearly the same SMD independent of flow rate between the limits of the particular design (see Section V.C.3).

A further need is for a device of variable spray angle independent of volume of flow. This again is difficult to achieve in any type of pressure atomizer without change of component parts. Variable spray angle can be obtained in the twin-fluid atomizer by simple adjustment while operating, and this method is commonly employed in the oil burning industry.

Many liquid mixtures now being sprayed in agriculture have a high content of solids. Hence, there is a demand for atomizers of greater energy transfer with larger orifices to deal with these more difficult mixtures.

A. GASEOUS FLOW IN NOZZLES

One of the characteristic features of the air-assisted spraying machine is that air is discharged from the mouth of a fan or blower casing into which insecticide, as a spray, is injected. In some of these equipments the function of a nozzle is not correctly understood.

The direction of air leaving the blades of different types of fan varies according to the blade shape. At a given speed the resultant velocity and direction of the air relative to the casing vary with the fan type. In general, the air direction can be either purely radial from the straight radial bladed type of fan or tangential to a circle smaller than the circle described by the vane tips in the forwardly or backwardly inclined vaned type.

Usually, air from such vanes is directed into a plenum chamber and redirected by being passed through a nozzle or nozzles. But in some types of spraying machine the fan casing itself is divided by a number of stationary vanes which provide openings to deliver the air in the form of a fan. In these cases, the spaces between these vanes do not perform the function of a nozzle but, rather, the vanes act as a radial diffuser; that is, they reduce the velocity of the air leaving the fan wheel periphery.

It is often not appreciated that the purpose and function of a nozzle are to convert the pressure energy in the gas to kinetic energy of flow by expanding it ideally. When a nozzle is in use it produces a reaction; it does so by accelerating the flow of gas and also imparting direction to it. The fact that a nozzle expands the gas and accelerates the flow infers a change in cross-sectional area with length, and with subsonic gas flow this change must be a convergence or, at least, not a divergence. It does not limit the shape of the cross section.

The nozzle imparts direction to the flow of the gas, and for this purpose it may have parallel walls after the convergence which produces acceleration. It can change the cross-sectional shape with length without necessarily changing the cross-sectional area—such as in the case of a fishtail or slot nozzle. Further, the nozzle emits a stream or column of gas as a jet, determined by the shape and dimensions of the mouth of the nozzle, flowing in a prearranged direction and moving in relation to the fluid surrounding the nozzle and nozzle mouth (usually the atmosphere), which surrounding fluid, at first stationary, becomes entrained into the jet. Thus, one characteristic of a jet of air discharged into the atmosphere is that, being surrounded on all sides by the air, it loses velocity progressively by friction with the atmosphere, and the atmosphere becomes entrained into the jet.

Figure 27 shows diagrammatically the velocity fields of a symmetrical subsonic jet, and there is little essential difference for sonic jets. The turbulent mixing region or free boundary layer commences as an annular ring around the nozzle mouth enclosing a conically shaped core of flow in which the velocity is uniform and equal to the initial velocity. At a certain distance, about five diameters, this core vanishes and the entire jet becomes a turbulent mixing region, in which the axial velocity decreases as the distance increases and all the velocity profiles are similar (Fig. 29*a*).

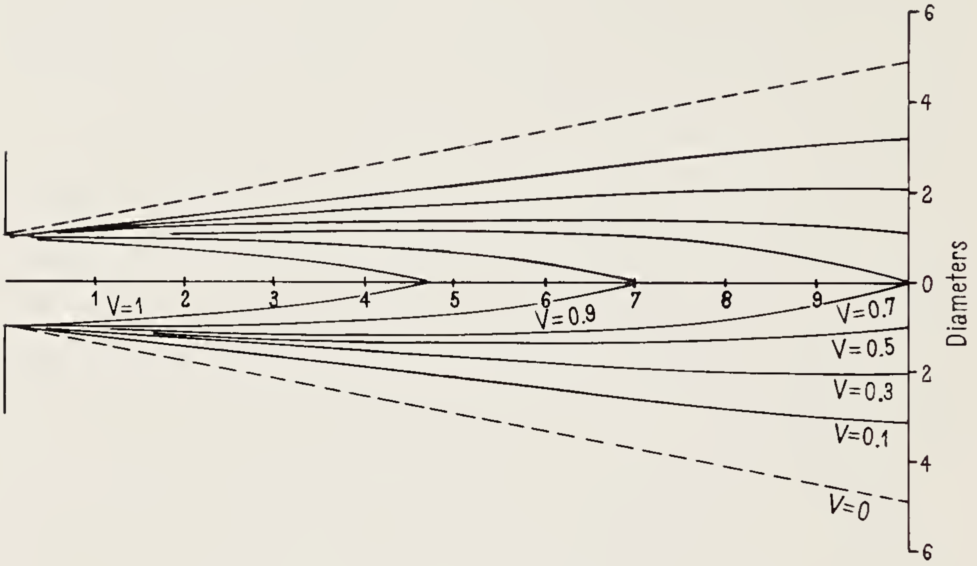


Fig. 27. Velocity fields of a symmetrical air jet.

The angle of expansion of a cylindrical jet is approximately constant at 12° half angle regardless of the diameter of the nozzle or the jet velocity, and thus the rate of fall of axial velocity with distance, expressed in nozzle diameters, is the same for all cylindrical jets. The gas velocity at any distance from the nozzle is thus directly proportional to the initial velocity and the nozzle outlet diameter. In consequence, at one pressure or velocity, the smaller the jet diameter the "shorter the throw," and, conversely, the greater the jet diameter (i.e., nozzle outlet diameter), the higher the air velocity at any given distance and the better the throw. For subsonic jets the effect of temperature difference is small. Lowering the jet temperature 60°C. below that of the surrounding atmosphere only increases the angle of divergence of the mixing region by 0.7%.

Figure 28 shows the decrease in axial velocity of a circular low-velocity air jet with distance, expressed in diameters of the orifice. The graph shows that the initial velocity is reduced to 40% at 20 diameters and to about 10% at 90 diameters. Potts and Garman (77) have measured the deposition velocities for mist blowers with circular outlets of different diameters and velocities, and these figures correspond more closely to the dotted curve and give about half the theoretical velocity at distances over 25 nozzle diameters.

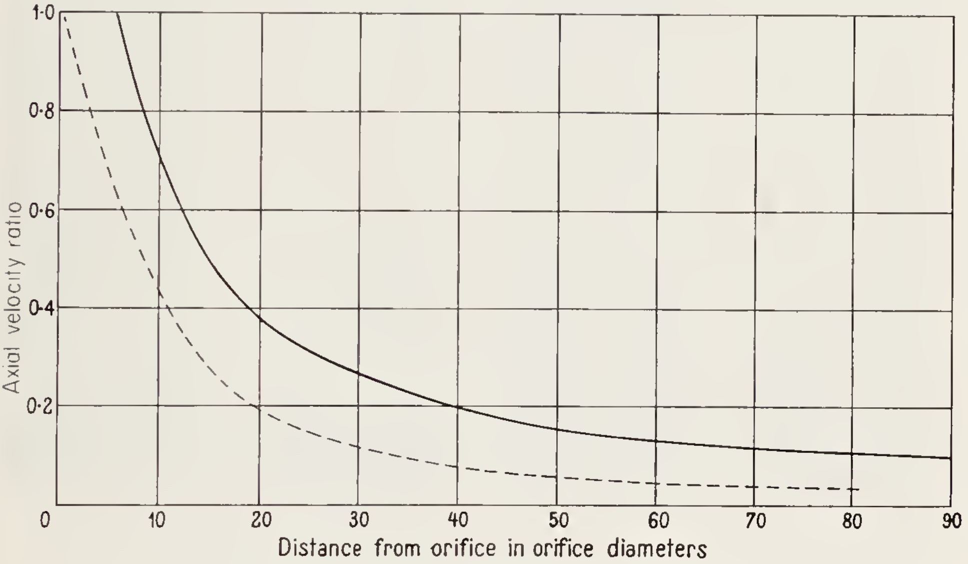


Fig. 28. Axial velocity of an air jet with distance.

With a given air capacity and static pressure from the fan, and using one nozzle, there is an optimum nozzle diameter and distance of throw. With agricultural equipment, therefore, the largest possible circular nozzle mouth should be used.

In agricultural practice it is very often desirable to distribute the air blast from the fan in the form of an arc. To direct the air in this manner it may be caused to issue from a slot or curved mouth fishtail as a sheet, the jet being effectively surrounded by the atmosphere on only two sides. Alternatively, the air may be made to issue from a number of short circular nozzles or pipes directing separate jets in an arc from a common plenum box. For a given air flow a small number of cylindrical nozzles can have greater diameters than a slot or fishtail width and thus achieve a greater throw. With common fan pressures and velocities below 400 ft./sec. it is undesirable and unnecessary to employ long tubes or nozzles. Short tubes of 1–3 diameters are usually quite adequate.

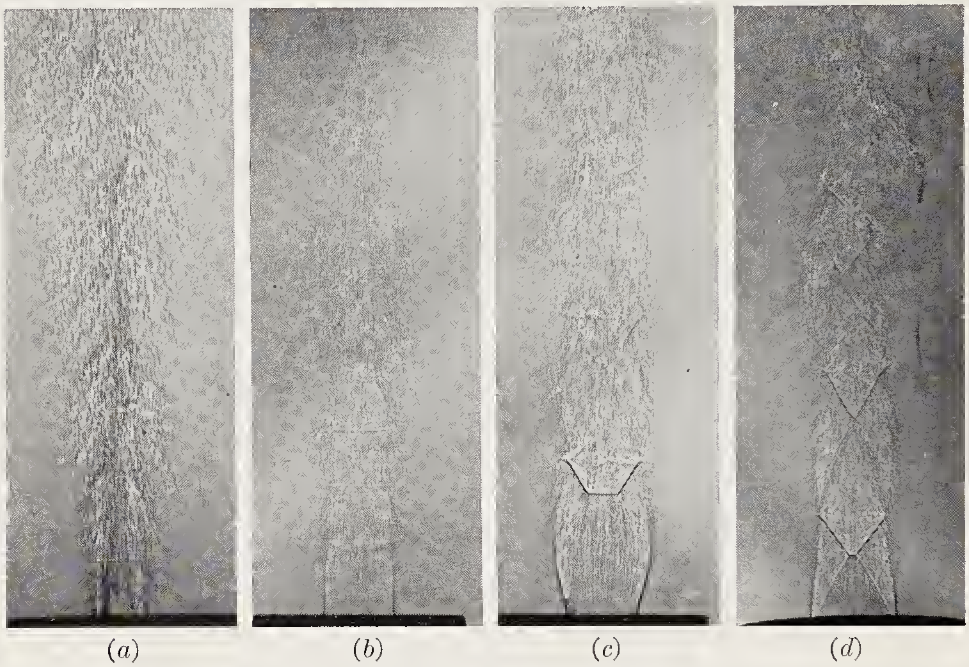

(*a*) (*b*) (*c*) (*d*)

Fig. 29. Gaseous flow through a sharp-edged orifice and nozzle: (*a*) orifice, 9 p.s.i., subsonic; (*b*) orifice, 20 p.s.i., sonic; (*c*) orifice, 100 p.s.i., sonic; (*d*) nozzle, 100 p.s.i., supersonic.

A supersonic jet having a higher velocity and lower temperature will give a greater penetration than a subsonic one. With a supersonic nozzle the mixing zone grows from the boundary layer inside the nozzle divergence. Thus the thickness of the boundary layer at the nozzle exit plane has an effect on the width of the mixing zone. If high penetration is required, a good interior finish should be used to keep the boundary layer to a minimum.

Figure 29*a*,*b*,*c* shows a subsonic, a "just sonic," and sonic jet issuing from a thin walled orifice having a 45° chamfer. Figure 29*d* shows a supersonic jet at Mach 1.6 flowing from a reservoir at 100 p.s.i.g. through a well-designed nozzle. The design characteristics of the supersonic nozzle are shown in Fig. 30.

Where it is desirable to deflect the air jet after it has been formed by the nozzle, it is necessary to use a vane in the jet or, preferably,

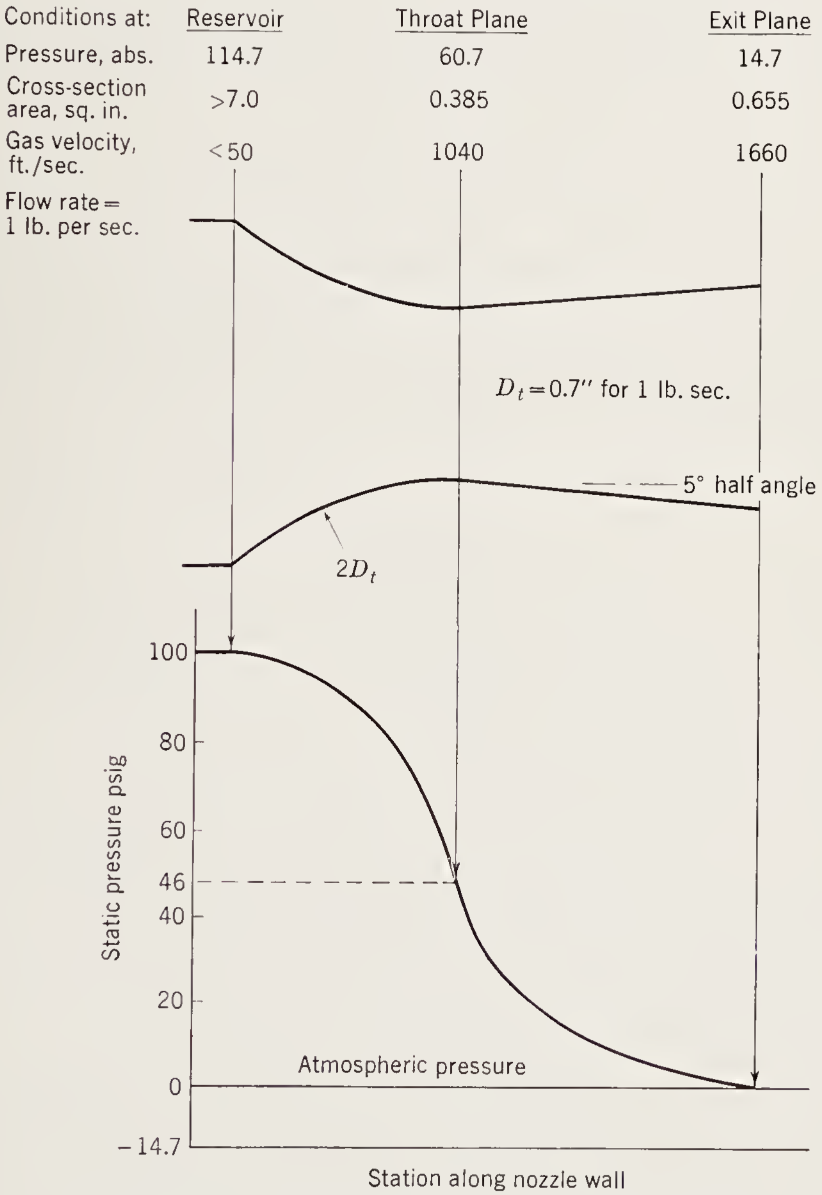

Conditions at:
Reservoir
Throat Plane
Exit Plane
Pressure, abs.
114.7
60.7
14.7
Cross-section area, sq. in.
>7.0
0.385
0.655
Gas velocity, ft./sec.
<50
1040
1660
Flow rate = 1 lb. per sec.
D_t = 0.7" for 1 lb. sec.
5° half angle
$2D_t$
100
80
60
46
40
20
0
−14.7
Static pressure psig
Atmospheric pressure
Station along nozzle wall

Fig. 30. The design characteristics of a supersonic nozzle.

a short tubular extension. Little difficulty arises in the use of these devices, but in the case of supersonic jets and deflection with a tubular extension may be greater than the tube angle (32).

1. Power Required for Moving Air

Where large volumes of air at low velocities are required for particle penetration, the fan horsepower can be very high. So also, the power required to move air in smaller jets at supersonic velocity for atomization can be very high. The horsepower per pound per second in a jet is given by

$$\text{hp.} = wu_e^2/2 \times 550 = 2.8233 \times 10^{-5}\, u_e^2 \tag{17}$$

Thus at sonic velocity, say, 1000 ft./sec., 28 hp. is required per 1 lb./sec. With a compression efficiency of 40% this becomes 70 hp. per lb./sec. At this figure about 1.4 hp. is required per 1000 cu. ft./hr. at 15 p.s.i.g. (1000 ft./sec.) or with fans at somewhat higher efficiency about 0.01 hp. per 1000 cu. ft./hr. at 0.107 p.s.i.g. (100 ft./sec.).

B. DISINTEGRATION OF DROPS TRAVELING IN A GASEOUS MEDIUM

Most spray nozzles of whatever type, working under practical conditions, result in a velocity difference between the liquid and the air, such that the most important mode of disintegration is that which occurs by gas inertia. This mode of disintegration can therefore be considered the final stage in all cases.

Two mechanisms of liquid breakup in an air stream have been considered. The first hypothesis is based upon survival diameter of large drops which disintegrate at a critical velocity difference, and the second hypothesis suggested by Taylor (101), is that one of the fundamental mechanisms of atomization of liquid in an air stream is the growth of unstable ripples on the surface of the liquid and the breakaway of fine droplets from their crests.

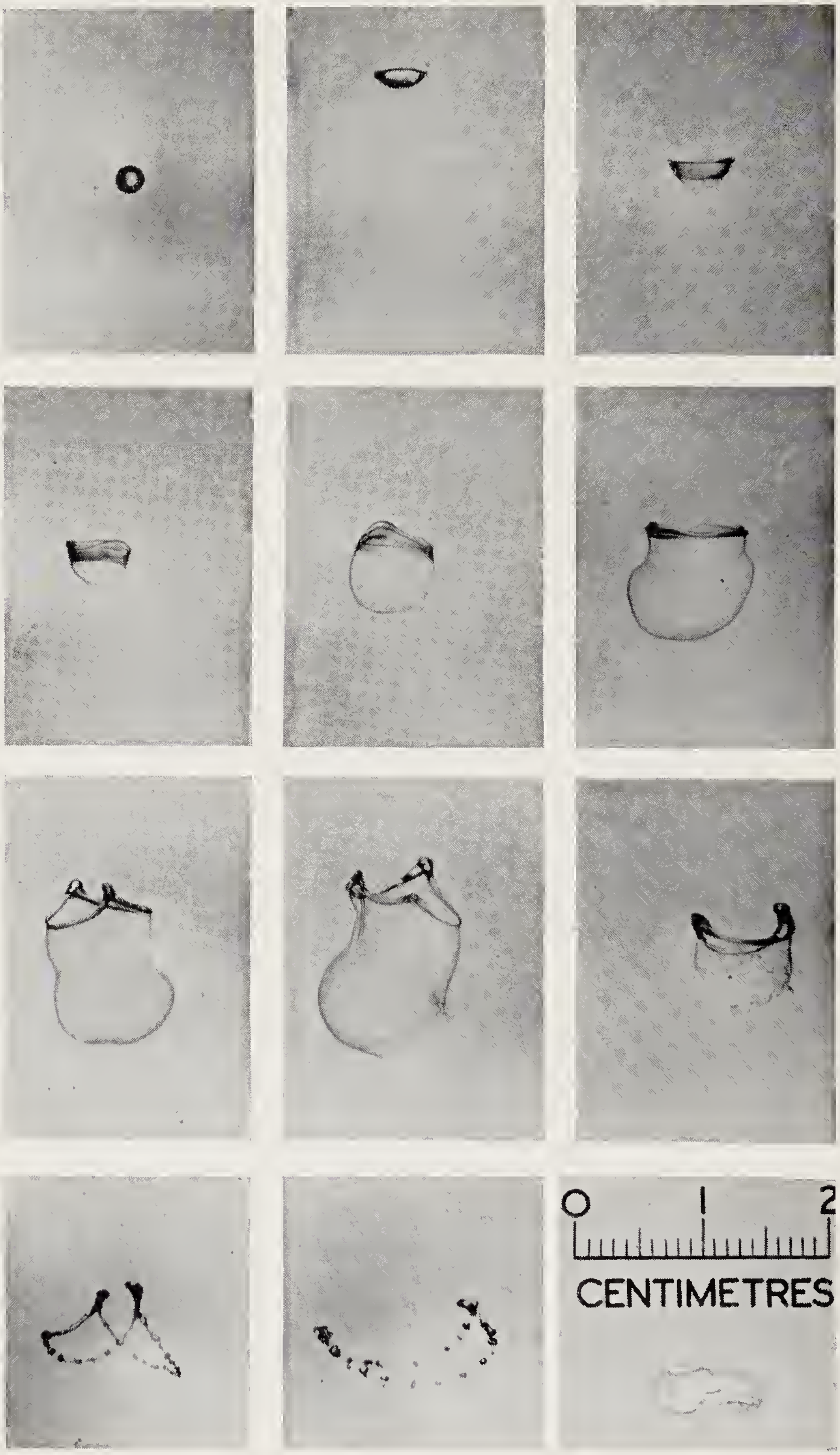
With drops in an air stream two types of breakup have been defined. (*1*) Where the liquid particles are large the unbalanced air pressure over the surface of the drop causes it to be blown into a bag, the thin film of which breaks up into small drops and the rim of which breaks up into larger drops. Lane (55) showed this process with larger drops of from 0.5 to 5 mm. diameter in a low velocity steady air stream (Fig. 31*a*). This bursting bag manner of breakup with relatively nonviscous drops only occurs over a narrow range of relative air drop velocity. It can be defined according to a critical Weber number of about 6 for low-viscosity liquids (48):

$$\mathrm{Wb}_{\mathrm{crit.}} = V^2 D\ \rho_g/\gamma > 13 \tag{18}$$

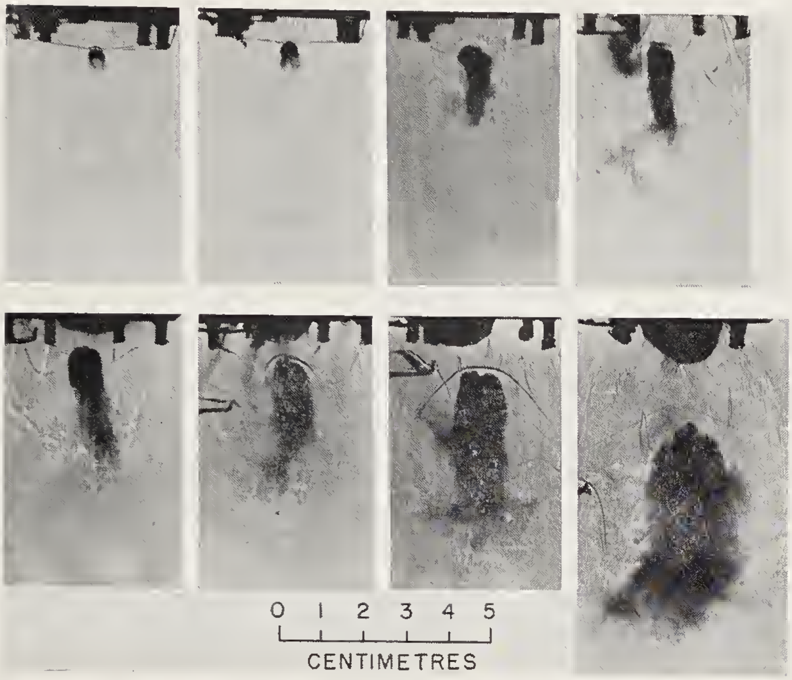
where V is the initial velocity difference of the liquid and gas leaving the atomizer; D is the diameter of the drop; ρ_g is the density of the gas or atmosphere; and γ is the surface tension.

(*2*) By studying the disintegration of smaller drops (1 mm.) in a transient air blast from a shock tube giving blast velocities up to Mach 2, Lane *et al.* (57) showed that when the air flow is fast and turbulent, breakup occurs in a different manner. In this case the drop becomes shaped like a saucer with its convex side facing the air blast, the edges of the saucer being blown out into a thin sheet from which threads and finally drops are formed (Fig. 31*b*). Taylor (101) deduced the shape of a drop while being accelerated, if it did not break up, as a planoconvex lenticular body of twice the drop diameter and this prediction is confirmed by the photographs. This second manner of breakup occurs at about Weber number = 30, and there is a transitional change over a range of conditions between these two types of breakup. In the first, disruption of the liquid surface occurs because the inertia stress developed by the surrounding gas exceeds the surface tension force opposing deformation of the drop. In the second, disruption occurs because of high shear stresses on the surface of the liquid particle which strip off layers of liquid from crests of waves on the surface before the bulk of the liquid can accelerate and be blown out into the bag-like shape. Lane and Green (56) showed that the drop is not shattered by the preleading shock front but only by the following high-velocity gas streams, and that during the shatter part of the drop is sufficiently coherent to offer enough obstruction to cause a shock front behind the drop cloud.

The velocities for breakup in a transient air blast are found to be lower than for a steady stream, this difference increasing for the smaller drops. A 1 mm. diameter drop in a steady air stream required 80 ft./sec. velocity, whereas in a transient blast it required only 52 ft./sec., a ratio of difference of about 0.65. This ratio of difference of about 0.7 is confirmed for all drops below 2 mm. Blanchard (5)


0
1
2
CENTIMETRES

(*a*)



(*b*)

Fig. 31. (*a*, facing page) Stages of breakup of water drops, 2.2 mm., in a low-velocity air stream, 24 m./sec. (*b*, above) Shatter of drops in a supersonic air stream (56).

showed that water drops suspended in an air stream were extremely sensitive to changes of velocity and could easily be caused to break up by momentarily varying the air velocity.

The relation for the limiting value of the relative velocity V corresponding to a critical drop diameter d below which shatter does not occur, is given by

$$V \propto (\gamma/d)^{1/2} \tag{19}$$

which, for water, becomes

$$V^2 d = 6.61 \times 10^6$$

where V is the velocity difference in ft./sec., and d is in microns within the range 500–5000 μ.

From the above equation this critical size for water at relative sonic velocity would be approximately 6 μ, but in practice drops larger than this have been observed to survive.

With the assumption that the above relationship holds for all drops below 500 μ, Fraser and Eisenklam (29) made a comparison of this critical diameter with the SMD obtained from several types of atomizer at different pressures and velocities. On the assumption that there is no air entrainment into the spray which will tend to reduce the relative velocity, they show that the larger drops will begin to shatter at a pressure of about 150 p.s.i. (150 ft./sec.) for a fan spray of FN = 2, and about 270 p.s.i. (200 ft./sec.) for the swirl spray, FN = 2, while the corresponding peripheral speed of the spinning disk is over 300 ft./sec.; that is, over 12,000 r.p.m. for a 6 in. disk.

TABLE VIII

Critical Drop Diameters for Sprays from Three Types of Atomizer

V, ft./sec.	Critical drop diameter, μ	Fan, SMD	Swirl, SMD	Spinning disk, main drop diameter
50	2650	309	310	412
100	661	197	153	206
150	294	181	135	137
200	166		129	103
300	73			69
500	27			
1000	7			

The instability of a viscous liquid to transverse waves interacting with the inertia of a fast-moving gas stream above it has been explored by Taylor (99,101), who determined the conditions for the most probable wavelength and the limiting conditions of viscosity. He suggests (*1*) that the most frequent drop size will be proportional to the most probable wavelength and that the constant of proportionality is near unity; and (*2*) that the drop size distribution function is proportional to the time factor of the amplitudes of the various wavelengths which have been assumed proportional to drop size. If this hypothesis is accepted, drop size at high liquid viscosity would be proportional to the relative velocity U to the power -1.33, diminishing down to U^{-2} for low liquid viscosity. In practice the drop size of twin-fluid atomizers varies roughly from U^{-1} to $U^{-1.5}$. Thus it appears the predictions of this theory are in qualitative agreement with experience. The two possible hypotheses mentioned at the beginning of this section, therefore, give nearly the same answer (81).

V. Drop Size in Sprays

One of the most sought after and most abused performance criteria of an atomizer is the size of the drops in the spray. Not only do atomizer design and the physical properties of the liquid and ambient spraying atmosphere influence the drop formation and dimension, but there are also transient effects to be considered which affect the drops' life history during flight, such as evaporation, coalescence, and secondary disintegration by air resistance. When quoting drop size results it is therefore necessary to specify the method of drop size determination and the ambient spraying conditions. Furthermore, a spectrum of sizes is obtained and its mean and size dispersion are of interest. For more precise information the drop size distribution ought to be given because the mean and the size dispersion do not give complete information of the size spectrum. The data which are available are generally limited to specific operating conditions and techniques of measurement, and much guess work and intuition are required to estimate results under different conditions. For these reasons accurate information is difficult to obtain and a superficial glance at spray data will very often give the reader a misleading prediction of a particular nozzle and atomizer.

Although present-day techniques of drop size determination allow a fairly accurate estimate of the drop size under most operating conditions, the actual requirements for most applications are only very vaguely known. Such ignorance has fostered the notion, which is firmly established, that the actual drop size required is often immaterial to the process, provided "good" atomization takes place, or provided the number of drops of smallest or largest size is controlled. Hence much of the effort which, in the past, has been directed toward determining the drop size from existing sprayers should now be directed toward establishing the drop size requirements for particular processes in which atomizers are used.

A. DROP SIZE DETERMINATION METHODS

The methods of drop size determination can be conveniently divided into two broad groups: direct and indirect. While the former require sampling from the bulk of the spray, the latter can be applied to the total spray without causing any interference with its development.

Direct methods consist of catching a representative sample of the drops on a target in such a way that shatter or coalescence is prevented and evaporation and diffusion are minimized. Limits of the method are that the size distribution may differ from one part of the spray to another, and thus the method of sampling may bias the result in favor of certain sizes. It is generally desirable to trap the drops in a liquid matrix, which is usually placed on a microscope slide, and the drops must survive impact and not shatter into smaller drops. The collecting liquid must necessarily be immiscible with the sprayed liquid, and it is also necessary that the drops remain round and do not coalesce or float on the collecting liquid.

Various mixtures have been prepared for collecting water and oil drops. For oils, a mixture of alcohol and glycerine is usually satisfactory, and for water a mixture of oil and aniline (30). It is also of importance that the drops do not evaporate in flight. This is prevented either by spraying liquids of low vapor pressure, below about 1 mm. of mercury at 15°C., or by employing a saturated atmosphere. A really satisfactory method of sampling sprays for microscope examination has not yet been achieved. Nukiyama and Tanasawa (75, 76) Doble (8), and Fraser and Eisenklam (29) carried out direct sizing of drops, and NIAE (72) used direct sizing from drops *in situ* in a greased matrix.

Five different techniques make use of the indirect method of size determination. *Photography* of the spray, or a portion thereof, appears to be the simplest technique, but although it has been applied extensively, it has not led to reliable results (40,59–62,87,104,113). The *photometric* technique deals with the change in the light intensity of a beam which either traverses the spray (86) or passes through the deposit on a transparent sampling slide. This observation can be related to a relevant mean diameter of the spray. The *sedimentation* technique has been applied to drops of kerosene in water or to solidified drops (90). In the *impressions* technique, records obtained by impact on a target are related to the sizes of the drops in flight, e.g., stains on blotting paper (13) or indentations on a silicone polished glass slide (4,72), on a film of magnesium oxide (35,66,67,75), on carbon soot (54,96), and on gelatine (84). The most frequently applied indirect method is the *freezing* technique. It consists of simulating relevant properties of a liquid by a substitute which is sprayed into an atmosphere at which the drops solidify after a conveniently short exposure. Wax sprayed into the atmosphere, which is often cooled by water sprays, is used to simulate oil (50); or oil is solidified in a liquid alcohol bath at a suitable distance from the nozzle (63); or water is solidified in liquid hexane (98). The sizes in the resulting product are determined by counting, sieving, filtration, or sedimentation.

Most drop-counting methods either by microscopic analysis or by automatic machines, such as that used by Marshall *et al.* (65), result in a drop size distribution showing the number of drops in suitable chosen size ranges. Analyzing these distributions and obtaining comparable unique parameters, such as mean drop sizes and dispersions, are problems which have not so far been satisfactorily solved because of the difficulty caused by variability of the actual size distributions obtained.

The applicability of a number of different distributions for particulate systems has been discussed by Fraser and Eisenklam (30) and Ranz (81). No drop size distributions have been found which follow accurately any of the distribution equations over a wide range. Hence the distribution parameters are best determined directly from cumulative volume per cent size curves. A graph of the size distribution, together with a calculation of a mean diameter and the size dispersion, constitutes the drop size analysis of each spray.

No practical absolute technique for standardized drop size measurement has yet been achieved. Rapid sizing methods have not yet been too successful, their accuracy being poor. For the sake of rapidity it may be necessary in the future to accept a lower accuracy, the data being then recorded as an apparent drop size based upon a standard test method.

Because there are a large number of means, it is extremely important that the kind of mean or median diameter is specified exactly. The two most commonly used are the volume or *mass median diameter* (MMD), which is the diameter dividing the mass of the spray (i.e., the volume) into two equal halves, and the volume *surface mean diameter* (SMD), which is the diameter of the drop having the same surface/volume ratio as the total spray.

In crop protection the MMD determines the effectiveness of the spray as measured by the amount of active material deposited per unit area, while the SMD defines the amount of exposed liquid surface, giving, in effect, a measure of the cover which may be obtained from a spray. So far the MMD has been used more widely for agricultural equipment. Quite evidently, it is not known which mean is the more relevant: in crop protection spray applications—whether the number of drops per unit area, the surface area of the spray particles, or the total mass is the more useful. A decision depends upon better biological data of the type of deposit required to control the pest.

Unfortunately these two means cannot be simply related for different types of drop distribution. Experimental values of the dispersion factor for Rosin-Rammler distribution lie between 2, 3, and 4, and the corresponding MMD/SMD ratios are 1.47, 1.20, and 1.12, respectively. In assuming a constant ratio of MMD/SMD, a constant dispersion of the drop size distribution must be assumed. Our work has shown that for fan, swirl, and twin-fluid atomizers of the vortex type the dispersion does not vary greatly from the average ratio of 1.28. Where original data quoted in this paper refer to MMD, the corresponding SMD quoted assumes the approximation MMD = 1.28 SMD.

B. RATE OF INCREASE OF LIQUID SURFACE BY ATOMIZATION

The rate at which atomization expands the liquid surface is very great. A sphere of 1 cm. diameter contains a volume of about $^1/_2$ cc. with a surface area of 3.14 sq. cm. If it is disintegrated into 10,000 equal spheres, each of them will have a diameter of 470 μ, approximately $^1/_2$ mm., and this process is coarse atomization. In general, the mean particle size range for atomizers is between 20 and 500 μ. Above this the apparatus is commonly defined as a sprinkler. The actual increase of surface area in the atomizing range is about 250–5000 times. Figure 32 shows the surface area plotted against drop diameter for 1 cc. of liquid. The very great rate at which the surface area increases below 50 μ diameter accounts for the fact that much greater energy has to be used to obtain the smaller particles.

C. DROP SIZE FROM DIFFERENT ATOMIZER DESIGNS

Three factors and their interactions affect drop size: (*1*) the atomizer design, which determines the way in which the liquid is discharged (see Section II); (*2*) the liquid properties, which influence the be-

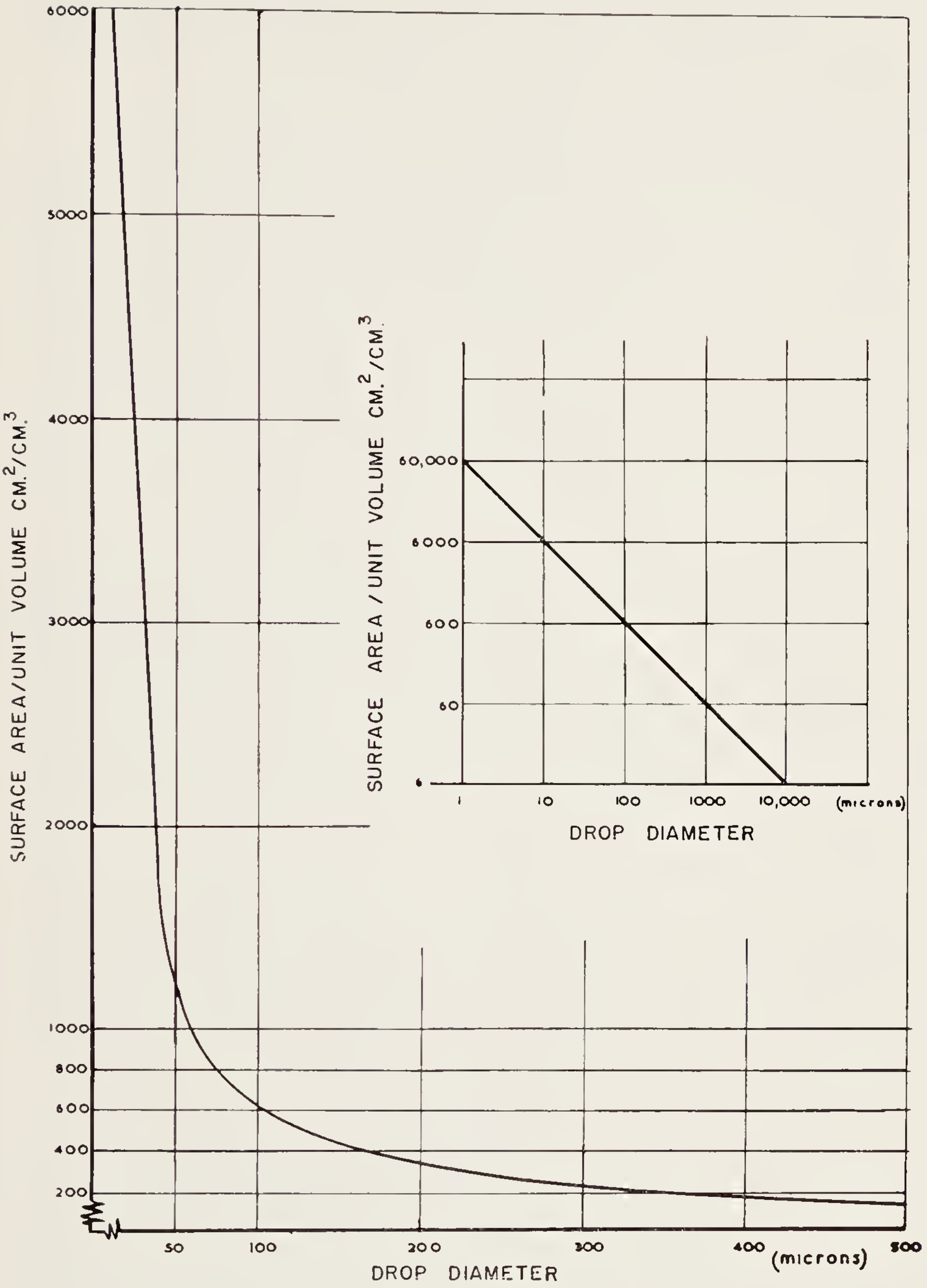


Fig. 32. Rate of increase of specific surface on reduction of drop diameter.

havior of the disintegrating sheet (see Section III.C); and (*3*) the spraying atmosphere, which affects the mode of disintegration (see Section IV).

It is sometimes stated that rotary atomizers give relatively large drops and good size uniformity; that pressure nozzles give drops somewhat in proportion to the size of the orifice diameter, usually above 50 μ, with medium uniformity; and that twin-fluid atomizers give drops smaller than other types, usually in the range of 15–50 μ, but have the poorest uniformity. This generalization is not true. Under practical working conditions of flow rate and velocity rotary atomizers behave very similarly to pressure nozzles; and at high pressures and velocities, which mean more disruption due to air friction, pressure nozzles give results more akin to a twin-fluid atomizer.

1. Pressure Atomizers

In pressure atomizers the drop size resulting from any liquid sheet depends on its thickness at breakdown and the physical properties of the liquid. Other than liquid properties, the factors, which determine the film thickness, and thus the drop size, are the liquid pressure, the ambient atmosphere, the spray angle, and the flow number (FN). FN, which is a convenient way of expressing the nozzle size and its throughput, is defined as follows:

$$\mathrm{FN} \propto C_Q A / \rho_L^{1/2} \qquad \text{or} \qquad \mathrm{FN} = Q/\Delta p^{1/2} \tag{20}$$

where C_Q is the discharge coefficient, A is the orifice area, ρ_L is the density of liquid, Q_L is the throughput, and Δp is the pressure differential. This number is conventionally quoted in units of "Imperial gallons per hour divided by the square root of the pressure in pounds per square inch." Hence for water

$$\mathrm{FN} = 293\ C_Q A\ (\mathrm{cm.}^2) \tag{21}$$

The flow number depends on the liquid density, and also viscosity, which affects it by influencing the discharge coefficient. In general, for each liquid, the flow number is independent of pressure differential once the spray sheet is fully established, and except for swirl spray nozzles it is practically independent of changes in viscosity; that is, it is unaffected by liquid temperature.

From dimensional analysis, it has been established (13) that the following relations between the properties of the liquid and the pressure atomizer generally hold:

$$\text{Spray mean diameter} \propto (\text{FN}\gamma/\Delta p\theta)^{1/3} \times (\rho_L/\rho_a)^{1/6} \tag{22}$$

where FN is the flow number of atomizer for a standard liquid, usually water; Δp is the pressure differential; γ is the surface tension of liquid; θ is the angle of fan spray sheet or sine of the half cone angle of a swirl spray sheet; ρ_L is the density of liquid; and ρ_a is the density of atmosphere.

Experimental results have also established that

$$\text{Mean diameter} \propto \nu_L{}^n$$

where ν_L is the kinematic viscosity in cs.; $n \sim 0.2$ hollow cone swirl spray nozzle, and $n \sim 0.1$ single orifice fan spray nozzle.

The above relation contains θ, a property of the spray which is not easily or quickly measured. Equations for drop size which are more readily applicable have been empirically determined and contain functions of the geometry of the nozzle only.

a. Swirl Spray Nozzles. Empirical formulas relating to liquid hydrocarbons of low surface tension have been derived by a number of workers using the method of spraying liquid wax to simulate the properties of a fuel oil and making a particle size analysis of the solidified wax by sieving (51,64,70,106). Recently Knight (53) has correlated a large number of drop size results obtained by these workers for nozzles of spray angle approximately 85° in the range of flow numbers up to about 4, as follows:

$$\text{SMD} = 220P^{-0.453}\,Q^{0.209}\,\nu_L{}^{0.215} \tag{23}$$

where Q is in lb./hr. This may be rewritten as

$$\text{SMD} = 335\,(\text{FN}^{0.209}/\Delta p^{0.348})\,\nu_L{}^{0.215} \tag{24}$$

within the experimental limits of: differential pressure, 12–300 p.s.i.; FN (flow number), 0.5–2.0; ν_L (kinematic viscosity), 2.0–18.5 cs.; spray cone angle, 80–90°; surface tension, 22 dynes/cm.; and liquid density, 0.746 g./cm.^{3}

An empirical expression obtained by Straus (95) using water and a small volume plug-type oil-firing nozzle (such as in Fig. 6*f*) is

$$\log \text{SMD} = 1.808 \times 6.94/\Delta p + 0.138\ \text{FN} \tag{25}$$

differential pressure, 25–200 p.s.i.; FN (flow number), 0.05–2.0; cone angle, ~ 60°; surface mean diameter > 70 μ.

With water, for a cone angle of 90°, this may be restated as

$$\text{SMD} = 437\,(\text{FN}/\text{P})^{1/3} \tag{26}$$

This equation is plotted in Fig. 33, and a typical drop size distribution at 100 p.s.i. is shown in Fig. 35.

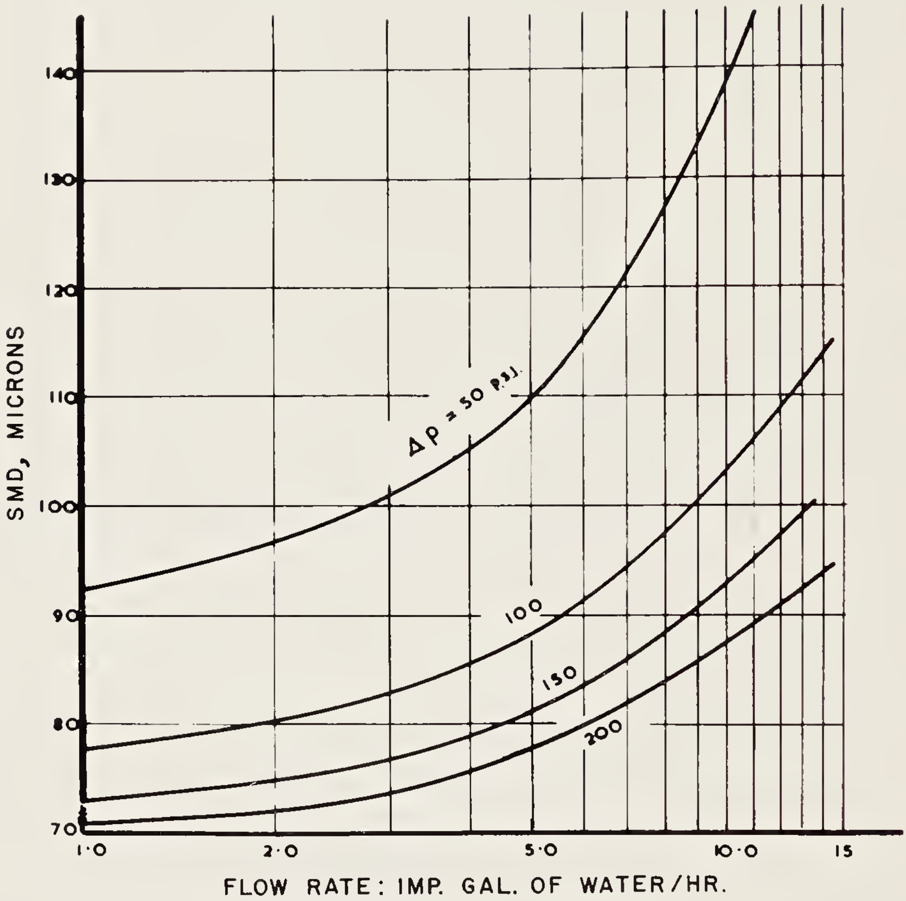


Fig. 33. Drop size from swirl spray nozzles with water.

Since the equation from the dimensional analysis, formula (22), contains FN/θ, which is a function of orifice diameter, attempts have been made to correlate drop size with orifice diameter for this type of nozzle. It has been shown experimentally that drop size is proportional to the orifice diameter between the limits $d^{1.0}$ and $d^{1.6}$.

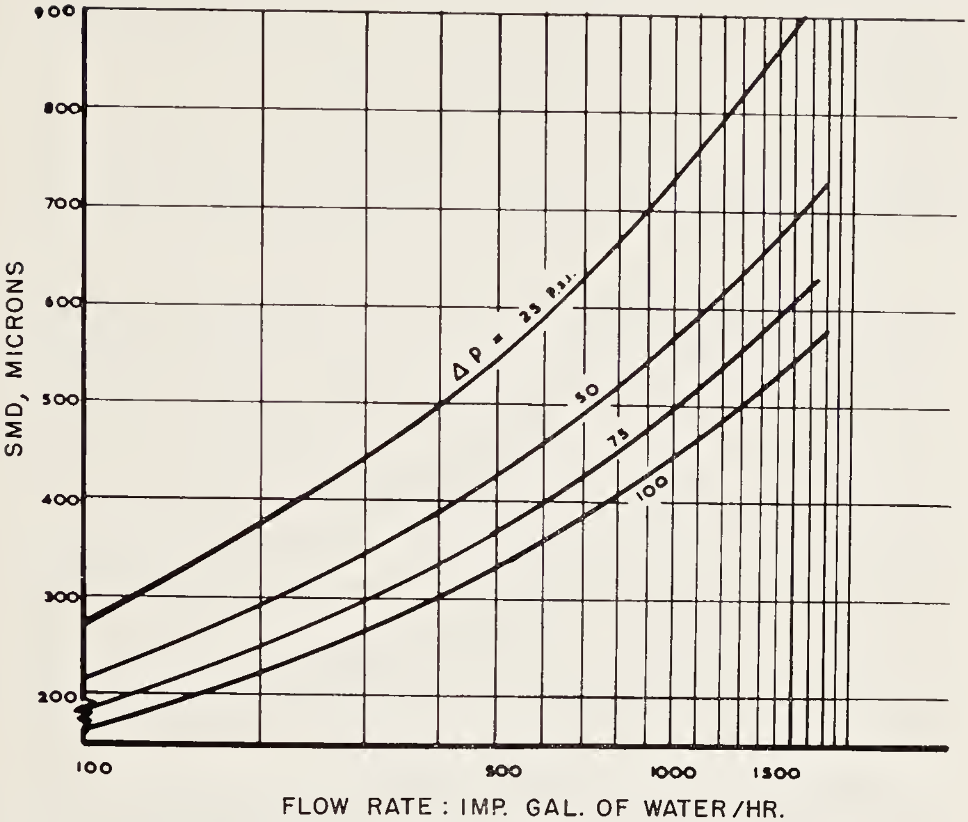


Fig. 34. Drop size from large swirl spray nozzles with water.

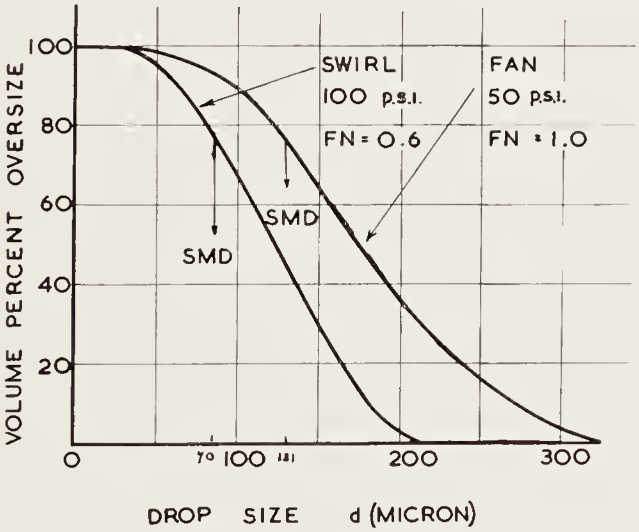


Fig. 35. Typical drop size distributions from swirl spray and fan spray nozzles.

Weinberg (111) found that for nozzles of very large flow rate of the swirl plate design, shown in Fig. 6*j*,

$$\text{SMD} = 119.2\ (\text{FN}^3/\text{P})^{1/7} \tag{27}$$

Δp, up to 100 p.s.i.; FN, 10–500; cone angle, approximately 85°. This equation is plotted in Fig. 34.

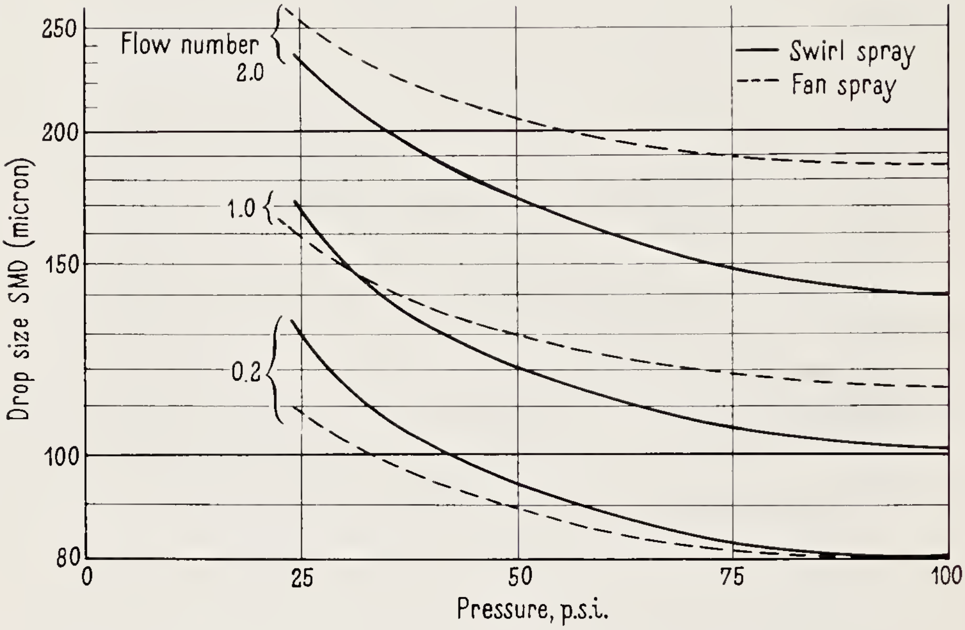


Fig. 36. Drop size from small swirl and fan spray nozzles with water.

b. Fan Spray Nozzles. It has been shown (12) that for fan spray nozzles of the rectangular orifice type (Fig. 2*b*), the discharge coefficient remains constant for a very wide range of liquid properties: viscosity from 0.4 to 81 centistokes, density from 0.80 to 2.1, and surface tension from 27 to 73 dynes/cm.

Straus (95) related the design characteristics of such nozzles to the properties of the sheet and to the drop size obtained on disintegration. He obtained an equation relating drop size to the velocity, length and thickness, and surface tension of the sheet. These results can be empirically expressed for water and a spray angle of 100–120°, pressures up to 100 p.s.i., and FN 0.35–2.0 as

$$\log \text{SMD} = 1.823 + (4.42/\text{P}) + 0.203\ \text{FN} \tag{28}$$

which for water at spray angle 90° becomes

$$\mathrm{SMD} = 585\ (\mathrm{FN}/\mathrm{P})^{1/3} \tag{29}$$

If the edges of the spray sheet are also considered, the SMD must be increased by 25%. Figure 35 shows a drop size distribution curve obtained from one of these nozzles of FN = 1, spraying water at 50 p.s.i.

The following equation for fan spray nozzles of the type shown in Fig. 2*b*,*c* was obtained by Dorman (13) by spraying dyed liquid horizontally from about 5 ft. onto filter paper and measuring the stain diameters

$$\mathrm{SMD} = 181\ (\mathrm{FN}\gamma/\theta\Delta p)^{1/3} \tag{30}$$

where θ is the fan spray angle in radians; γ is the surface tension, 28–73 dynes/cm.; Δp is 45–105 p.s.i.; FN is between 0.5 and 8; and liquid density is between 0.8 and 1 g./cm.^{3}

Equations (25) and (28) are plotted in Fig. 36 so that the drop sizes for fan and swirl spray nozzles can be compared for similar pressures and flow numbers. It should be noted that for low flow numbers both types of atomizers behave similarly, but at high flow number a swirl spray nozzle gives a smaller drop size, particularly at higher pressure. With both atomizers atomizing water an increase of pressure beyond 100 p.s.i. does not materially reduce the drop size.

Impact nozzles. So far very few drop size determinations have been made with this type of nozzle. Rasbash (82) obtained a limited number of results with three nozzle sizes at two pressures. These nozzles had an impact plate at 30°. Sampling was carried out about 5 ft. from the nozzle, and the water drops were caught in oil. He obtained the following equation:

$$\mathrm{MMD} = 1480\ (\mathrm{FN}/\Delta p)^{1/3} \tag{31}$$

where Δp was 10–100 p.s.i., and FN was 0.38–5.2. Assuming MMD = 1.28 SMD, this gives an empirical equation for water

$$\mathrm{SMD} = 1160\ (\mathrm{FN}/\Delta p)^{1/3} \tag{32}$$

With impinging jet nozzles of a 90° angle, using water and sampling in a similar manner to the above, the following equation is obtained:

$$\mathrm{MMD} = 630\ (\mathrm{FN}^{0.235}/\Delta p^{0.265}) \tag{33}$$

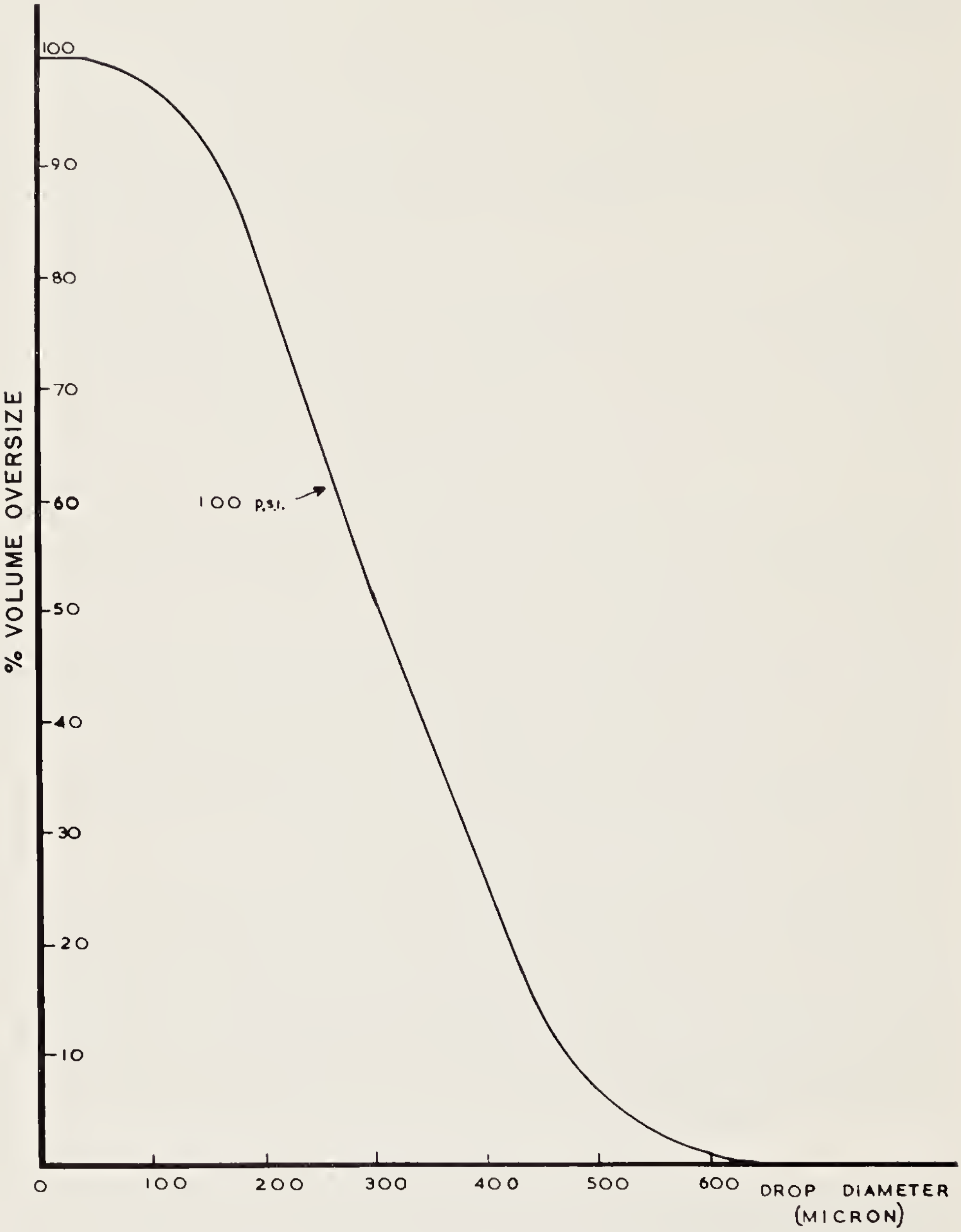


Fig. 37. Typical drop size distribution for an impinging jet nozzle with water.

where the differential pressure is 5–85 p.s.i., FN for both jets is 0.8–8.5, diameter of the flow pipes is $^1/_{64}$–$^1/_{16}$ in., and discharge coefficient is 0.73. Figure 37 shows a drop size distribution curve for an impinging jet nozzle of 55°, FN = 6 at Δp 100 p.s.i. atomizing water.

Simple jets. Merrington and Richardson (68) found that for orifice diameters greater than 2 mm. the drop size is independent of the diameter and therefore independent also of FN. Again assuming the SMD/MMD ratio above, their empirical formula for water can be rewritten as

$$\text{SMD} = 10{,}000\ (1/p^{1/2}) \tag{34}$$

c. The Efficiency of Pressure Nozzles. Most of the energy applied to the liquid in a pressure nozzle is converted to kinetic energy in the drops and subsequently to the atmosphere by entrainment, and only a small fraction to surface energy. Thus the mechanical efficiency as judged by the production of surface energy is low. Figures given by Giffen and Muraszew (36,37) are shown in Table IX for a swirl spray nozzle of FN 0.9. It is noticed that the efficiency is very low even for low-viscosity liquids and that, although it falls further with increasing viscosity, viscous loss is not the controlling factor.

TABLE IX
Efficiency of a Swirl Spray Atomizer (36,37)

	Water	Fuel	Lubricating oil
Viscosity, cs.	1.01	1.8	32.0
Surface tension, dynes/cm.	72.0	23.9	32.0
Pressure differential, p.s.i.	135	144	152
Surface mean diameter (SMD), μ	89	86	112
Efficiency, %	0.53	0.17	0.16

2. Rotary Atomizers

It is commonly thought that spinning disks produce more uniform sprays than other atomizers, but at comparable throughput pressure and disk speed the sprays have much the same size distributions. The suggestion that rotary atomizers give more uniform drops is due to the findings with small disks at low feed rates where the mechanism of drop formation from the edge of the disk is similar to that observed at the end of a stationary circular tube (see Fig. 39*a*).

Harkins and Brown (41) derived a relation for the formation of drops from a tube connecting the drop size to the diameter of the tube and the physical properties. By replacing the gravitation acceleration by centrifugal force, the following formula is derived

$$d_M \omega \, (D \rho_L / \gamma)^{1/2} = \text{constant} \tag{35}$$

where d_M is the diameter of the main drop in microns, ω is the angular velocity, and D is the diameter of disk in cm.

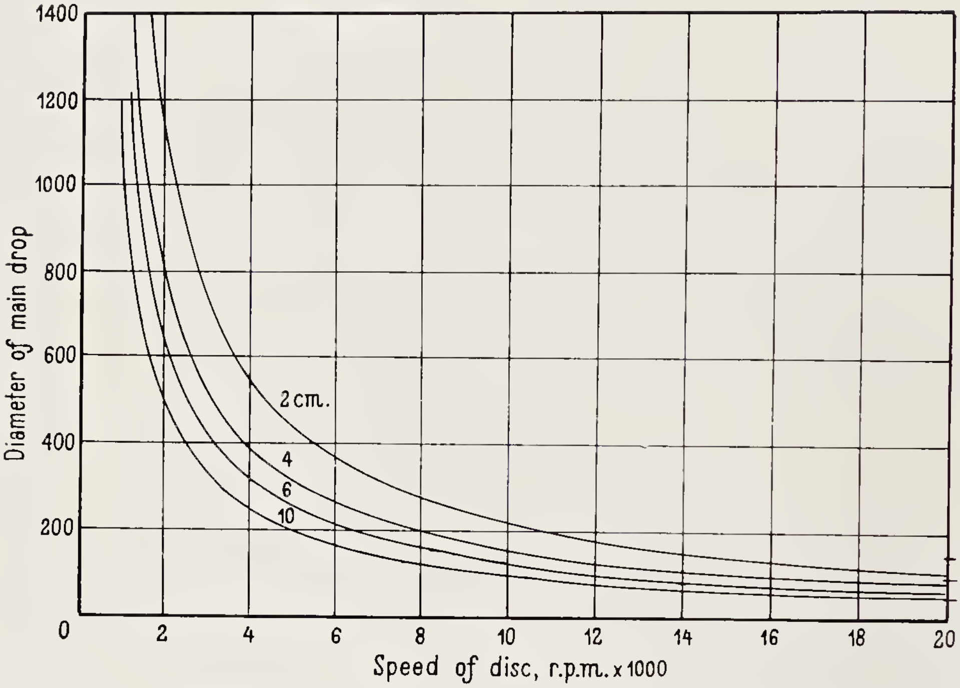


Fig. 38. Main drop sizes from spinning disk atomizers with water.

This equation was experimentally verified by Straus (95) and the constant found to lie between 3.68 and 3.86 with a mean of 3.76. This result is the same as that obtained by Walton and Prewett (105). The drop size equation can be rewritten

$$d_M = (360{,}000/S) \, (\gamma / D \rho_L)^{1/2} \tag{36}$$

where S is the disk r.p.m., γ is the surface tension in dynes/cm., and ρ_L is the density in g./cm.^{3}. This equation is plotted for different speeds and disk diameters in Fig. 38.

Experiments with other than square edge profiles indicate that the profile has a distinct influence on the drop size. A 45° beveled profile, for instance, reduces the main drop diameter in equation (36) by

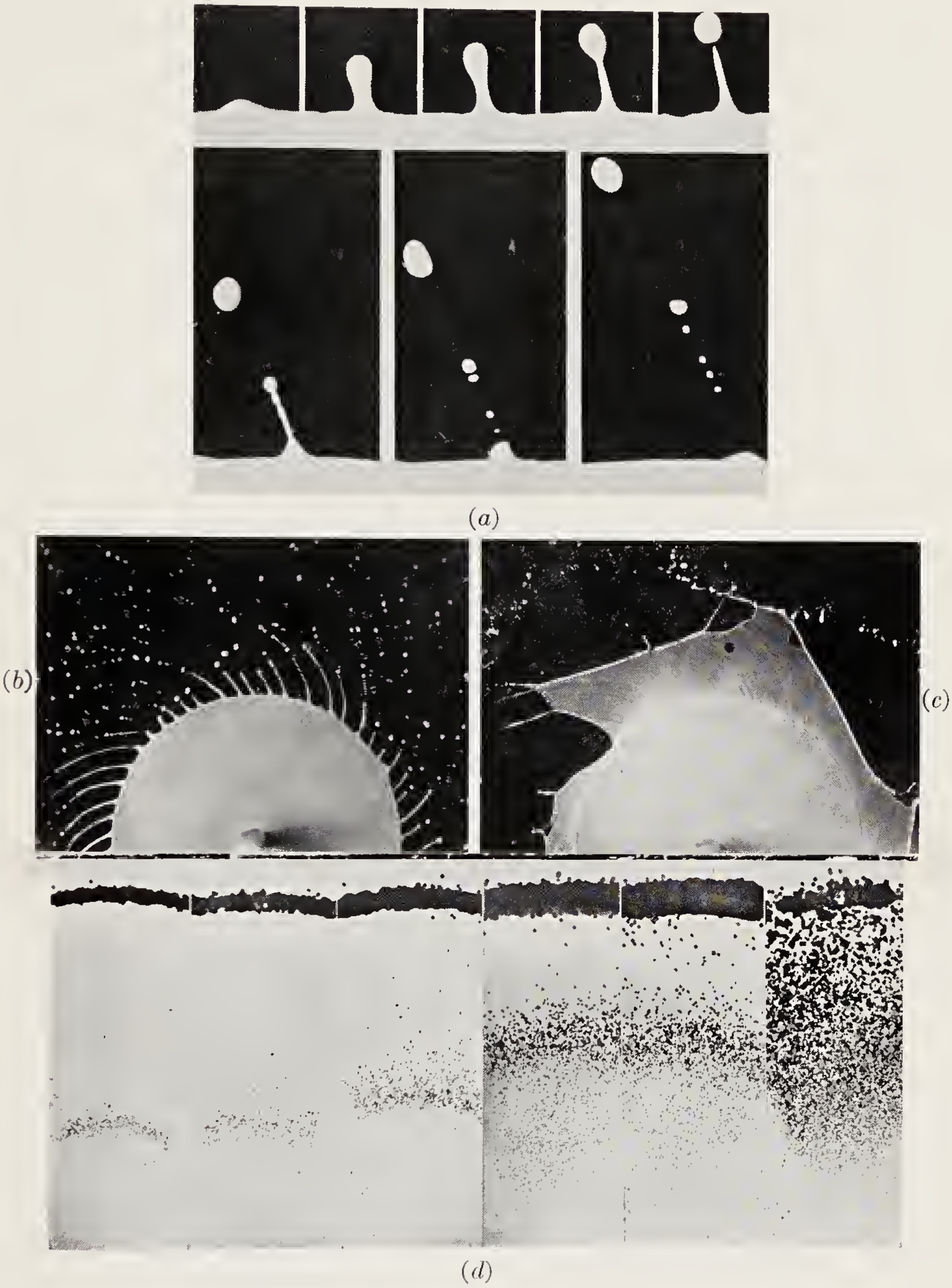


Fig. 39. Disintegration of a liquid by a small spinning disk with increasing flow rate: (*a*) eight successive instances of drop formation at low flow rate, 2.33 cc./sec.; (*b*) threads formed at the disk periphery at 15 cc./sec.; (*c*) sheet formed at disk periphery when flooded at 24 cc./sec.; (*d*) drop separation by gravity from a spinning disk, 4 cm. diam., 1000 r.p.m., at low feed rates, 0.3, 1.6, 3.2, 9.5, 14.3, and 22.2 lb./hr.

13%. It is also important to note that the conditions of a uniform main drop size only exist when the drops are formed at the rim of the disk between the upper and lower edge and there is a critical flow rate range that produces such conditions.

Small disks working at low feed rates can be made to produce homogeneous sprays when the main and satellite drops are separated in sizes by gravity. The degree of separation that can be obtained is illustrated in Fig. 39*d*, which shows the drops collected from a disk of 4 cm. diameter at 1000 r.p.m. spraying methyl salicylate at between 0.3 and 22 lb./hr. (105).

At practical feed rates drops may be formed from long ligaments away from the rim or from a sheet of liquid in the atmosphere outside the disk perimeter, as mentioned in Section III. Furthermore, air entrainment around the disk in this case plays a part in the disintegration. Adler and Marshall (2) studied a large number of different designs of disk at constant feed rate and peripheral velocity and showed that the drop size distribution did not change for various designs of disk. Herring and Marshall (46) found it possible to develop a correlation for drop size distribution in terms of feed rate and disk speed. Figures 40 and 41 show two performance charts for estimating the effect of peripheral speed and feed rate on drop size distribution with vaned disks.

Although formulas relating drop size to the operating conditions vary among workers, one formula which correlates data over a wide range is

$$\mathrm{SMD} = 4.2 \times 10^4 (1/N)^{0.6} (1/\rho)^{0.5} (\eta m/D)^{0.2} (\gamma/y)^{0.1} \qquad (37)$$

where N is the rotational speed in r.p.m. (range 860–1180 r.p.m.); ρ is the liquid density in lb./cu. ft. (62.4–88); η is the absolute viscosity in centipoise (1–9000); m is the mass flow rate in lb./hr. (33–4050); D is the disk diameter in ft. (0.17–0.67; 2–8 in.); γ is the surface tension in dynes/cm. (74–100); and y is the wetted periphery in ft., where y equals πD for disks or nb for vaned disks: n is the number of vanes, and b is the height of vanes in ft.

The above equation shows that the length of the wetted perimeter has a small effect, the most important factors influencing drop size being rotational speed and diameter.

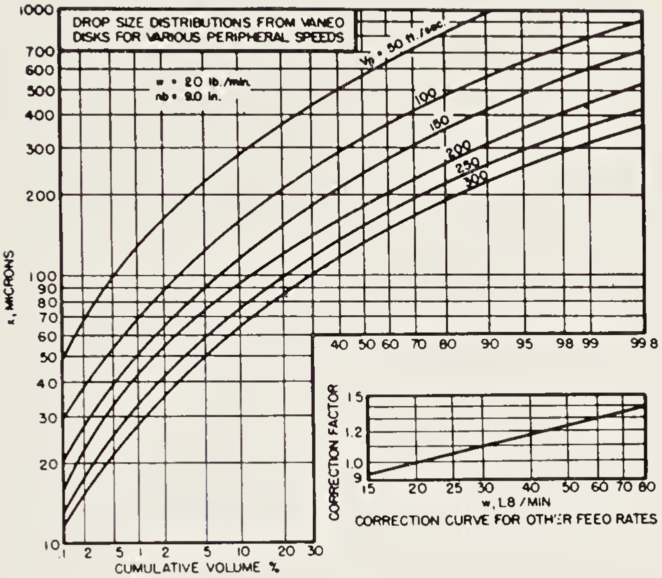


Fig. 40. Performance chart for vaned disk atomizers showing effect of disk peripheral velocity on drop size distribution (65).

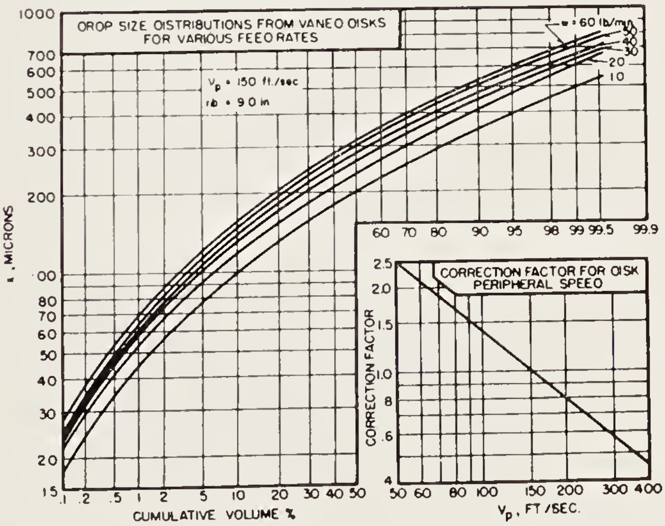


Fig. 41. Performance chart for vaned disk atomizers for various feed rates (65).

3. *Twin-Fluid Atomizers*

The effects of liquid physical properties on drop size from twin-fluid atomizers have not yet been well established. Experimental work with small blast nozzles of the internal mixing type operating with high volumetric air/liquid ratios led to the development of the following formula by Nukiyama and Tanasawa (75) applicable within the range of liquid physical properties given:

$$\mathrm{SMD} = \frac{585}{V}\left(\frac{\gamma}{\rho_L}\right)^{1/2} + 597\left(\frac{\eta}{\sqrt{\gamma\rho_L}}\right)^{0.45}\left(\frac{1000}{Q_L/Q_A}\right)^{1.5} \tag{38}$$

where γ is the surface tension in dynes/cm. (range 30–73); η is the viscosity in poise (0.01–0.3); ρ_L is the liquid density in g./cm.^{3} (0.8–1.2); V is the relative air/liquid velocity in m./sec.; and Q_L/Q_A is the liquid/air volume ratio (free air). SMD is in microns.

For water this equation can be expressed as

$$\mathrm{SMD} = (16{,}400/V) + 39.4\,(M_w/M_A)^{1.5} \tag{39}$$

where V is the velocity of air relative to liquid at the nozzle in ft./sec., and M_w/M_A is the water-air ratio in lb. with air at 65°F. Their original work was confined to subsonic relative velocities, but Bitron (4) has confirmed it for supersonic velocities.

The formula shows that the drop size is determined by the relative fluid velocities and their mass ratio. Thus drop size is reduced as the air pressure and the air/liquid ratio are increased. To obtain a surface mean diameter below 20 μ, air velocities greater than 700 ft./sec. and air/liquid weight ratios above 10 lb./lb. may have to be employed. It is interesting to note that if sonic velocity and an infinite air/liquid mass ratio are assumed for liquids of low viscosity, we can eliminate the last two terms in equation (38). Thus the minimum particle size for water would be

$$\mathrm{SMD}_{\mathrm{min.}} = (585/336) \times 8.7 = 15\ \mu \text{ approx.} \tag{40}$$

and for oils with lower surface tension, between 8 and 10 μ.

Wetzel and Marshall (112), experimenting with venturi injection-type atomizers and using molten wax, density 0.83 g./cc., viscosity 9 centipoise, surface tension 29.5 dynes/cm., expressed their results as

$$\mathrm{MMD} = 4.2 \times 10^6\,(V_a - V_L)^{-1.65} \times D_0{}^{0.35} \tag{41}$$

where MMD is the mass median diameter (50% volume oversize), V_a is the velocity of air stream in ft./sec., V_L is the velocity of liquid jet in ft./sec., and D_0 is the diameter of liquid jet in in. Their results show good agreement with the Nukiyama and Tanasawa results when wax was used and the air velocity was above 400 ft./sec. (about 2 p.s.i.). But no agreement was obtained when spraying a high density molten alloy, indicating that the Nukiyama-Tanasawa equation should be employed only within the range of liquid properties for which it was established and should not be applied to liquid metals of high surface tension where nonspherical drops are produced (see Fig. 22*f*).

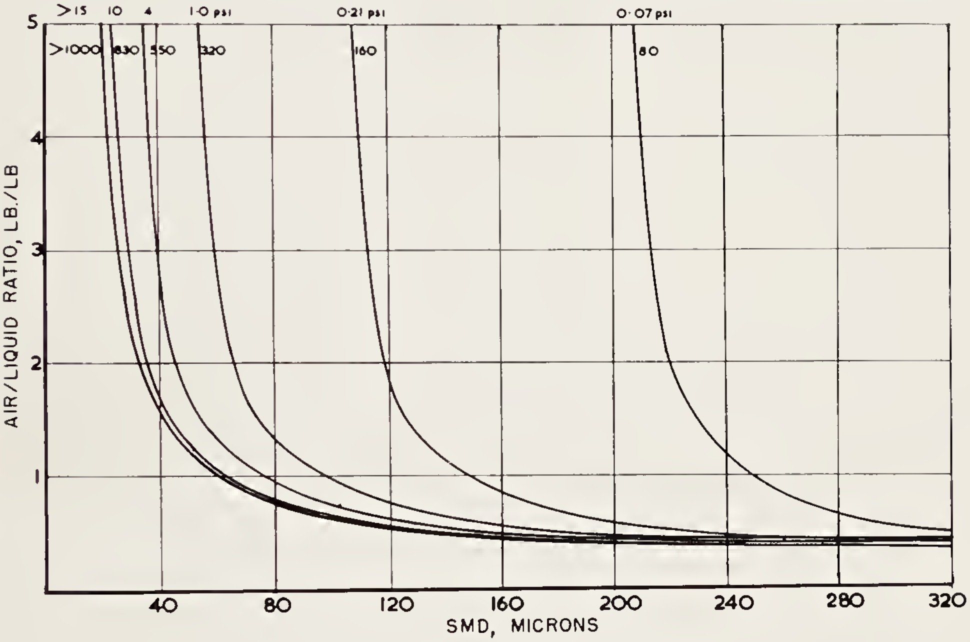


Fig. 42. Relation of air/liquid ratio and drop size for various gas velocities. Pressures in p.s.i. and velocities in ft./sec. appear at top of graph.

Figure 42 illustrates the relation of air/liquid weight ratio to SMD for variations of gas velocity according to formula (38). It is observed that considerable atomization is achieved with relatively low air pressures, less than 1 p.s.i. But to achieve drop sizes with water below 80 μ requires increasing quantities of air and, also, a greatly increased velocity difference. The rate at which the energy has to be increased to obtain the velocity to reduce the drop size is shown in Fig. 16.

The effect of increasing viscosity utilizing the same formulas is shown in Fig. 43 where each curve is for a velocity of 830 ft./sec., corresponding approximately to 10 p.s.i.g. The viscosities are 1, 10, and 100 cs. with a surface tension of 28 dynes/cm., compared with water 1 cs. and 76 dynes/cm. The comparatively great effect of increased viscosity with the same gas velocity is observed, but formula (38) may only be valid up to about 40 cs.

It is interesting to note that although there is much variation in design, fluid pressure, physical properties, and method of drop size measurement, the SMD given by several practical types of this kind of atomizer can be correlated reasonably well when plotted against air/liquid ratio, as shown in Fig. 44 and Table X. The correlation again illustrates the large expenditure of air required to reduce the SMD below 40 μ with any design or viscosity compared with that required to obtain SMD = 100 μ. The curve A is that given by the Nukiyama-Tanasawa equation (38) using a simple injection atomizer with a liquid having viscosity 10 cs., density 0.94 g./cm.^{3}, and surface tension 28 dynes/cm. atomized at an air pressure of 10 p.s.i. Curve B is calculated from a determination of Courshee *et al.* (7) using the simple in-

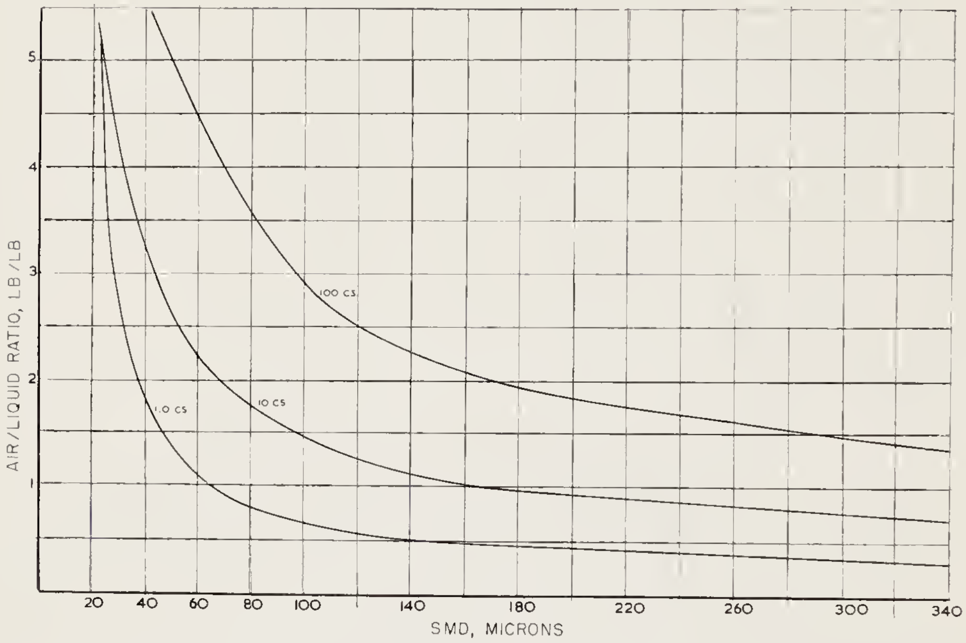


Fig. 43. Relation of drop size to air/liquid ratio for liquids of different viscosities.

jection atomizer of the type shown in Fig. 12*b*. These curves are in contrast to the band representing the results from more modern designs of both internal and external mixing types, where rotating streams of air are usually employed. Even so a very large mass flow of air is required at a pressure usually exceeding 15 p.s.i to achieve mean drop sizes of 20 μ or below. Curve C shows the results from a nozzle of the type shown in Fig. 12*f* and curve D as for Fig. 12*k*. The curves show a practically constant drop size with variation of flow rate at a constant air pressure of 10 p.s.i.

Where the liquid mass flow is very small and the gas velocity can be sonic, a mean drop size less than 10 μ may be obtained with a gas/

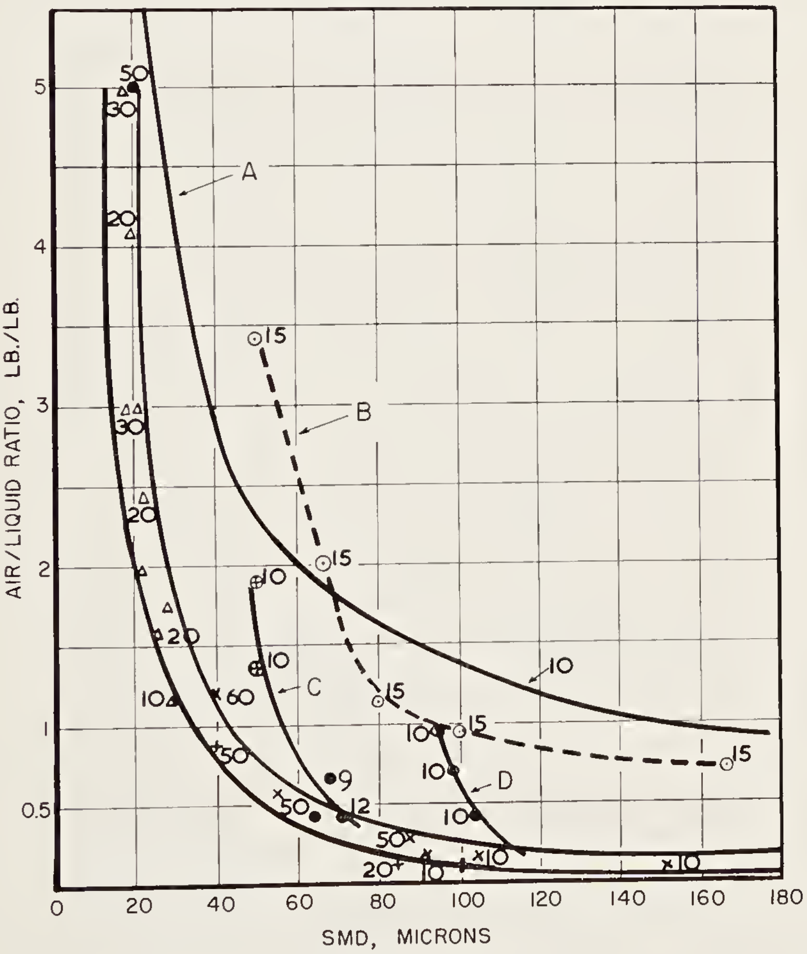


Fig. 44. Drop size correlation for twin-fluid atomizers.

TABLE X

	Symbol and reference						
	Δ (33)	× (79)	+ (79)	⊕ (23)	● (23)	○ (23)	⊙ (7)
Type of atomizer	Internal mixing	Internal mixing (Fig. 12*g*)	Internal mixing	External, mixing	Vortex cup (Fig. 12*f*)	External mixing vortex cup (Fig. 12*k*)	External mixing, simple injection (Fig. 12*b*)
Liquid delivery range, lb./hr.	Up to 0.115	54–269	86–218	50–280		166–400	9–59
Liquid used	Petroleum ether	Wax	Wax	Wax	Water	Wax	Water
Liquid properties							
Viscosity	0.69	40	20	40	1.0	40	1.0
Density	0.734	0.93	0.9	0.93	1.0	0.93	1.0
Surface tension, dynes/cm.	20[a]	23[a]	23[a]	23[a]	73	23[a]	73

[a] Approximate.

liquid ratio of 3 lb./lb. or more, and this appears to be possible regardless of design. Where the liquid flow rate is high, however, and small drops are desired, the design of the atomizer becomes important,

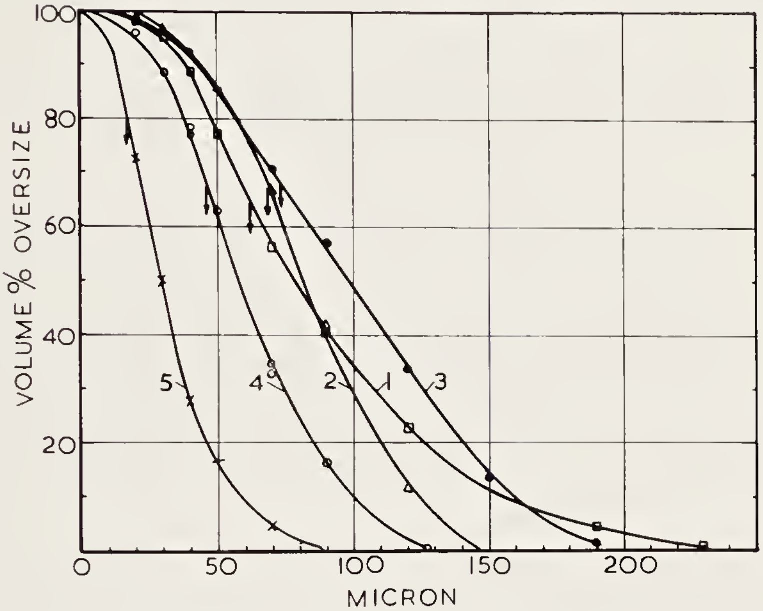


Fig. 45. Drop size distributions for water from twin-fluid atomizers of the vortex cup type (as Fig. 12*f*). Vertical arrows indicate SMD.

TABLE XI

	Curve number				
	1	2	3	4	5
Air annulus, thous.	50	50	50	50	50
Pressure, p.s.i					
Air	9	9	8.5	16	35
Water	50	22	40	10	10
Water flow rate, gal./hr.	26.5	17.7	11.4	5.9	5.9
Air/water ratio	0.465	0.69	1.05	3.0	5.1
Surface mean diameter, μ	63	69	74.5	47	18
Sampling distance, in.	9	9	12	36	36

for it is necessary to ensure that the liquid is prefilmed and the contact of the liquid and gas are made more efficient.

The drop size distribution of twin-fluid atomizers will change with nozzle design and size and, also, by adjustment of component parts in any one design. Drop size distributions for an external mixing vortex type atomizer (as Fig. 12*f*) are shown in Fig. 45 and Table XI. Differences are observed with change of air/liquid ratio, pressure, annulus width, and sampling distance. A smaller SMD is obtained when the sample is taken near the atomizer because of loss of small particles by drift and coalescence of particles with distance.

VI. Spatial Dispersion and Deposition of a Spray

The operation of spraying a surface, either vertically or horizontally, consists essentially in moving the nozzle in vertical or horizontal sweeps, the spray bands being so arranged that there is a slight overlap so that no surface is untreated. In hand spraying in the field it is found that the operator can usually judge quite accurately if he is spraying squarely onto a surface and, also, with practice the speed at which he is moving the nozzle. But it is much more difficult to keep constant the small distance of the nozzle from the surface. Similarly, with a mobile spray boom, the speed and squareness to the ground are controlled but the ground contour causes the small spraying distance to vary greatly. One of the requirements, therefore, of a lancehead or spray boom is to give the same spray pattern over as wide a range of working distance as possible and, since the number of overlappings should be limited, as broad a spray as possible.

Coulter and Ellis (6) determined the quantity of spray deposited by spraying a $2N$ solution of sodium carbonate from a moving atomizer onto a nonabsorbant vertical surface and titrating so that a true measure of the quantity of deposited insecticide was made. The surface consisted of a number of glass slides so that the variation of deposit relative to the nozzle could be estimated. It was shown that when coating a surface with spray from low-volume nozzles, 1–15 gal./hr., the deposition efficiency is low. The surface may be coated unevenly and a high percentage of the spray can be lost before it reaches the surface.

The uniformity of spray deposition from any nozzle varies with target distance. The amount of spray deposited per unit area at right angles to the spray axis can be plotted against the distance of that area from the spray axis and thus a family of "deposition curves" is obtained.

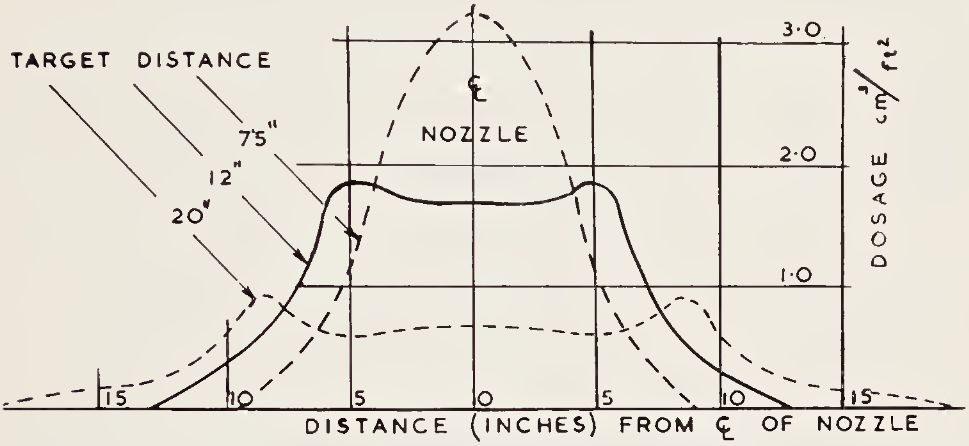


Fig. 46. Deposition curves from fan spray nozzles (as Figs. 2*b*, 4*b*). Differential pressure 30 p.s.i.

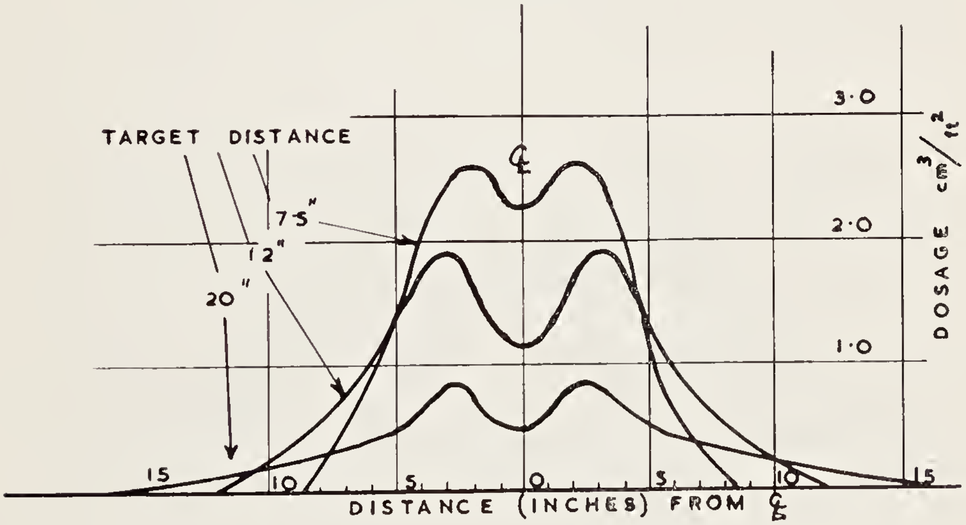


Fig. 47. Deposition curves from an open cone swirl spray nozzle (as Fig. 6*f*) Differential pressure 30 p s.i.

Such curves, for fan spray nozzles of the type shown in Fig. 2*b* show a high deposition near the axis at close working distance, which falls rapidly with distance from the axis. As the target distance is increased, the high deposition falls and the width of the spray band increases, and uniformity improves (Fig. 46). With an open cone swirl spray nozzle deposition is low at the axis with two maxima on either side, which is in contrast with fan sprays but, again, at increased distance the deposition becomes more uniform (Fig. 47).

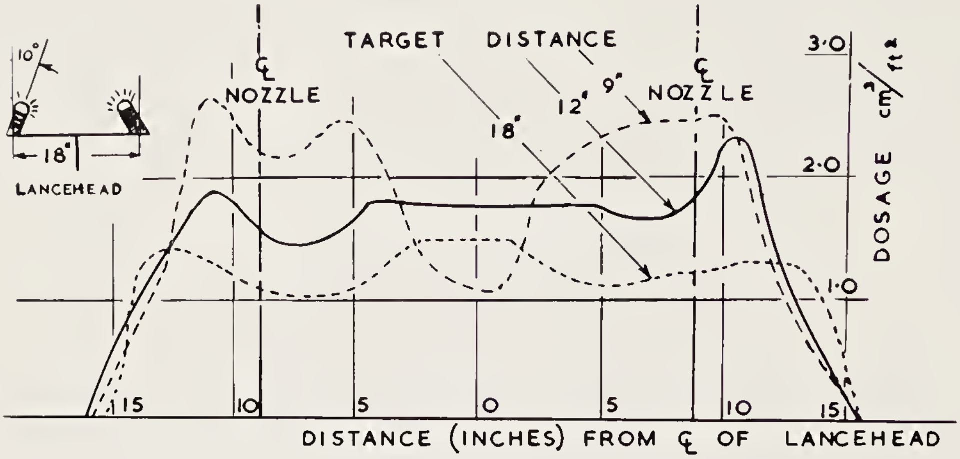


Fig. 48. Deposition curves from a fan spray lancehead using two nozzles (as Fig. 2*b*). Differential pressure 30 p.s.i.

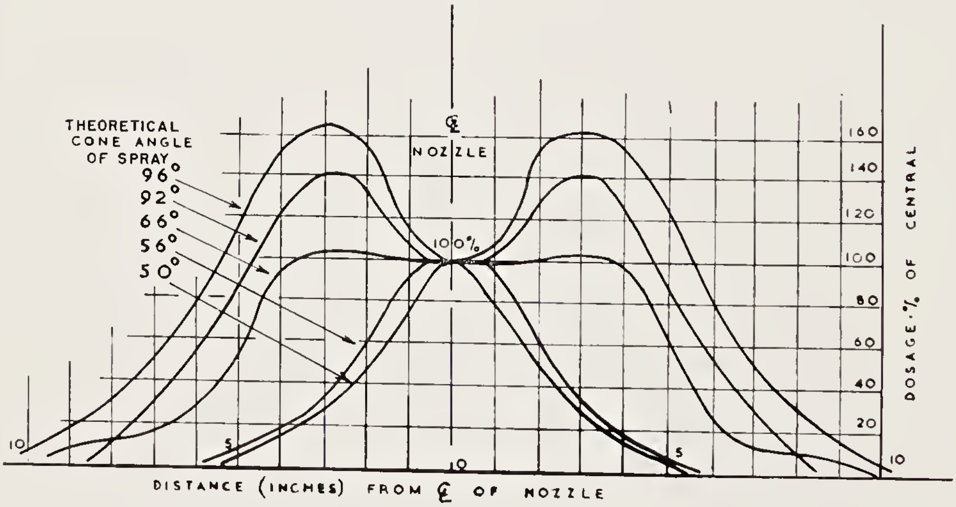


Fig. 49. Deposition curves from swirl spray nozzles of varying spray angle. Differential pressure 50 p.s.i., target distance 12 in.

For hand spraying equipment nozzles with low spray angles, 30–

60°, are most suitable. The use of two nozzles on a lancehead arranged at such an angle that the spray boundary of the combination is at right angles to the sprayed surface will give a constant band width irrespective of target distance. If the target distance and the distance between the nozzles are equal, say 2 ft., then the spray angle required will be 45°. If wider spray angles are employed, then the distance between the nozzles must be increased, and generally, because it is difficult to design satisfactory pressure nozzles with spray angles less than 30°, the distance between the nozzles should not be less than 14 in. Figure 48 shows the improvement in spray band width and deposition from two fan spray nozzles mounted on a lancehead 18 in. apart and inclined inward at 10°.

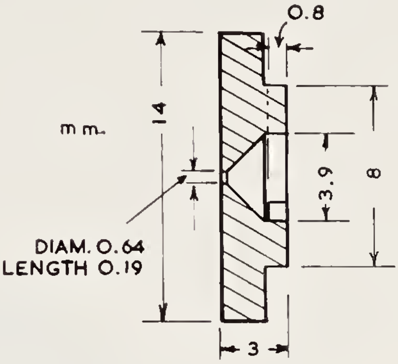


Fig. 50. A design of swirl spray nozzle for a lancehead giving a solid cone spray.

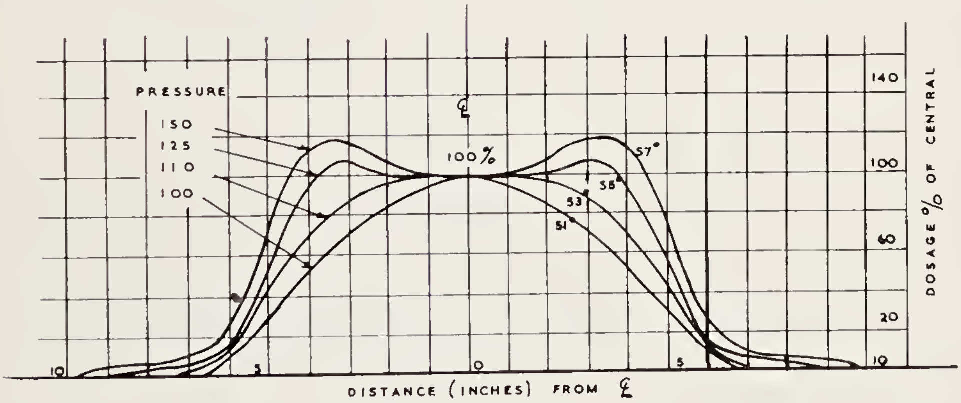


Fig. 51. Deposition curves from a swirl spray nozzle (as Fig. 50).

From the point of view of uniform deposition a swirl spray nozzle operating with no air core giving a drowned spray is generally preferable to a hollow cone. Somewhere between the two designs there is evidently a more ideal deposition curve, and this is found to be given by nozzles having spray angles of 55–65°. Figure 49 shows deposition curves from swirl spray nozzles with different spray angles operating at the same pressure (50 p.s.i.) and target distance (12 in.).

Although theoretically the most uniform deposition and constant band width are given by a pair of nozzles of spray angle 45° inclined inward at $22^1/_2°$, it is found in practice that the spray contracts toward the axis (see Figs. 8 and 24) so that the spray angle should be greater than 45°. The best compromise appears to be given by 55°.

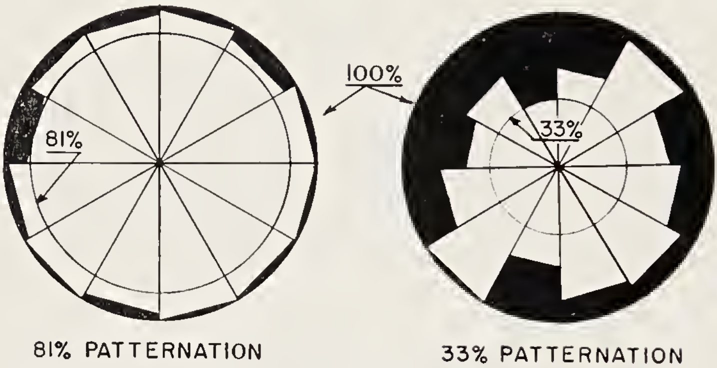


Fig. 52. Patternation diagrams from swirl spray nozzles.

Figure 50 shows the design of a swirl spray nozzle giving a drowned or solid cone spray for use on a lancehead where a pair of nozzles will deposit a minimum dosage of 2 cc. of water per sq. ft. at a traverse rate of 2 ft./sec. (20 gal./acre), and a spraying distance of 24 in. The nozzles are thus mounted 24 in. apart inclined inward $22^1/_2°$. The nozzle throughput based on a 70% deposition efficiency is 5.7 cc./sec. or 4.5 Imp. gal./hr. The design dimensions are: spray angle, 55°; D_2, 0.64 mm.; D_3, 3.9 mm.; A_s, 0.025 cm.^{2}; l/D_2, 0.3; D_3/D_2, 6.0; Δ, 1.01. Figure 51 shows the characteristic deposition of this nozzle at varying pressures.

To obtain a satisfactory result with any nozzle it is important that the uniformity of the spatial dispersion of the spray be measured. Machining errors, blockages, and damage to the tip of the nozzle can seriously affect the uniformity. The test most frequently carried out for this purpose is the so-called "patternation test," which consists of spraying into a segmented trough having 12 sectors. The volumetric deposition in each sector is measured and the results can be plotted so that the area in each sector is proportional to the corresponding deposition volume. The maximum volume is denoted by 100%, and the distribution is expressed as the maximum percentage collected in any one sector. Figure 52 shows the comparison of a good nozzle with a patternation of 81% and a bad one of 33%.

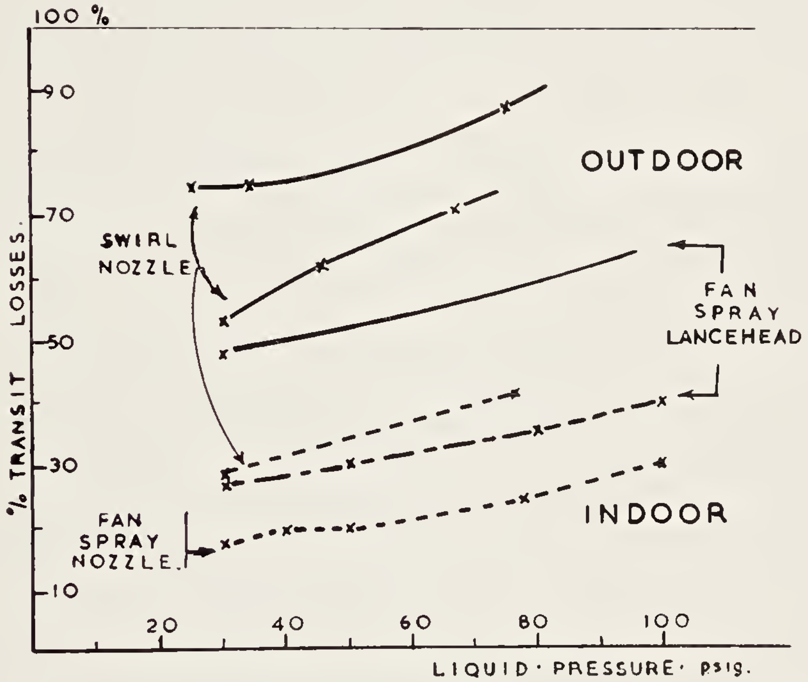


Fig. 53. Spray transit losses at target distance of 12 in. with swirl and fan sprays.

Transit losses in spraying are high owing to both drift and evaporation of small particles. With nozzles of small flow rate (FN 0.1–2) and working distance greater than 18 in., there is a very serious loss of spray in transit; as much as 70% of the spray may be lost when spraying a vertical surface 2 ft. from the nozzle. This loss can be reduced by using a lower operating pressure and the shortest possible working distance, but even for a comparatively efficient nozzle operating at 30 p.s.i. in still air a 17% loss at 12 in. is obtained and a 35% loss at 18 in.

The loss in transit is still further increased under the ambient conditions commonly encountered in the field, where spray drift is increased by wind. Figure 53 shows the comparative losses measured for small swirl spray and fan spray nozzles, spraying at a distance of 12 in. In all cases there is an improvement at lower pressure but a very great difference between the losses when spraying indoors and outdoors. Fan sprays tend to give lower losses than swirl spray nozzles because of the larger particle size at the same pressure.

Clearly, means to moderate or remove the effect of wind and the use of an optimum drop size combined with the removal if possible of all very small drops would achieve much greater efficiency and economy.

In a spraying machine the most vital component, the spray nozzle or atomizer, may cost only a thousandth part of the cost of the whole machine, yet the performance of this small part is paramount in achieving the desired result and more and more importance will, of necessity, have to be given to it. In the future a more specialized atomizing device must be considered by the user, not merely as a terminal orifice of a spraying lance or boom, but as a mechanical instrument to be preserved and removed from the machine during inactive periods. With a more intelligent approach to the vital importance of the atomizer itself, the future holds the possibility of using devices which may give reasonably accurate variations of mean drop size.

With our improving knowledge, which has enabled us to determine spraying and biological inefficiencies, it might be argued that because field conditions appear uncontrollable and our present operating efficiency is low we should continue with present methods. This is a policy of despair. Conversely, it can be reasoned that present inefficiencies must lead to new inventive steps brought about by future research.

Acknowledgments

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INNATE TOXICITY OF FUNGICIDES

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CONTENTS

I. Introduction 107
II. Metal Ions 110
    A. Uptake of Copper by Spores (Early Studies, 1892–1903) 110
    B. Uptake of Copper, Mercury, Silver, and Cerium by Corn Smut Spores (1922) 112
    C. Quantities of Copper and Mercury Required to Inhibit Germination of Smut Spores (1927–32) 113
        1. Factors Affecting the Uptake of Mercury 114
    D. Uptake of Copper by Conidia of *Monolinia fructicola* and by Other Species 115
    E. Presence of Toxicants within Spores and Other Cells 118
    F. Determination of Innate Toxicity by Radioisotope Techniques . . 119
        1. Silver and Mercury 120
        2. Cadmium and Zinc 123
        3. Cerium 123
        4. Nonidentity of Receptor Sites for Some Toxicants 124
III. Sulfur 124
IV. Organic Toxicants 126
        1. Glyodin Base 126
        2. Dichlone 126
        3. Dithiocarbamates 127
        4. Other Organic Toxicants 127
V. Permeability of Spores and Distribution of Toxicants within the Spores . 128
VI. Interpretation of Dosage-Response Curves 129
VII. Comparison of Efficacy of Fungicides with Other Biocides 131
References 131

I. Introduction

For a toxicant to be able to prevent the germination of fungus spores it must come in contact with and inhibit a process necessary for such germination to proceed. In general, one would expect this to take place inside the spore or at least on the spore membrane. Interference with germination could also result if the solution surrounding the spore were exceedingly toxic to the germ tube about to emerge and thus prevented its continued growth, while not exerting any toxic effect on nongerminating spores. This has been suggested as a possible means by which spores are inhibited. In general, it is often assumed by workers in this field that the fungus spore is difficult to penetrate by toxic materials. However, a large amount of evidence is accumulating which indicates very clearly that movement of material into and out of spores takes place much more readily than has previously been supposed.

In order to know more about how toxicants act it is important to know the actual quantities of various toxicants taken up by spores under conditions which produce inhibition or lethality. It is the purpose of this review to report results that have been obtained by various workers on this phase of fungicidal action. In discussing uptake of toxicants various authors speak of adsorption, absorption, or merely sorption to describe this process, and some perhaps are quite convinced that either adsorption or absorption accurately describes what is involved. In this review these terms will be more or less avoided and the expression uptake will be largely used to designate the removal from solution by fungus spores of toxicants in which they are suspended. The phenomena involved most certainly usually include adsorption, absorption, and chemical reactions. Movement of materials out of the cells as well as uptake of toxicants also takes place.

In studying toxic effects in fields other than that of such very small organisms as fungus spores or bacteria, it is customary to determine the quantity of toxicant required per unit weight of organism to bring about the response desired. Toxicity is therefore commonly expressed as the LD_{50} value, or the quantity in milligrams per kilogram required to kill 50% of the organisms. In special situations it is sometimes necessary for practical purposes to know the concentration of a toxicant in the air or in an ambient solution which will exert a given effect. However, in these instances the true dose based on the weight of organism being affected is not known. The organism may and probably does accumulate the toxicant with time, since under these conditions a large reservoir of toxicant is available.

With organisms which can be handled as individuals a definite amount of toxicant can be applied and the mean quantity required to produce an LD_{50} value can be determined readily and simply. However, with very small organisms heavy reliance has been placed on the concentration of the applied solution as a measure of the toxic effect, with very little emphasis on, or even a disregard of, the relative quantities of organism and toxicant present. When organisms such as fungus spores are suspended in a toxicant solution, the amount available, or the amount able to exert its effect on the spores, may vary from none at all, indicating complete exclusion of the toxicant from the spores, to very large quantities in instances in which the conidia accumulate large quantities of the toxicant presented to them. The true dose thus remains entirely unknown in such tests.

It has been known for over 50 years that fungus spores can take up large amounts of toxicants from dilute solutions. Various authors over this period have pointed out the importance of toxicant uptake. In spite of this, relatively little emphasis has been placed on the determination of LD_{50} or ED_{50} values (15) for fungicides based on the weight of toxicant taken up per unit weight of fungus spore, although this concept has received emphasis by students of insecticidal action (44).

Methods for determining toxicant uptake require quantitive determinations of relatively small amounts of the toxicant chemicals. While this offers some difficulties, the comparative lack of information on innate toxicity is more probably the result of a lack of emphasis on the importance of this information. The fact that the commonly used methods for determining the toxicity of bactericides and fungitoxicants are based on external concentrations only has also no doubt helped to bring about lessened emphasis on innate toxicity.

The problem is further complicated by the fact that many of the effective and commercially important fungicides are relatively insoluble and tests are commonly carried out with large excesses of toxicant remaining undissolved. When a series of concentrations is tested in this way, despite the excess of insoluble chemical, the values usually fall in line on a dosage-response curve (37). However, it certainly remains doubtful how such large excesses of insoluble toxicant, almost always exceeding the spores in mass, can enter quantitatively into the toxic responses noted. As early as 1924 Bokorny (8) stressed the fact that the determination of the external concentration required to inhibit a fungus does not give adequate information on inherent toxicity. He stated that a definite amount of poison is necessary for a definite amount of living substance. He recommended that in practice both the lethal dose and the activity of different dilutions of disinfectants be determined.

The data available on uptake of chemicals by spores of various species are widely scattered as to time and the journals in which the results have appeared. Prior to 30 years ago there was considerable interest in the uptake by smut spores of mercury and copper, and to a lesser extent of silver, cerium, and other ions (5–7,67,73). This work was carried out in detail and with care, and some of it has been repeated more recently. As a result of the greater availability of radioisotopes, and by the use of other analytical methods, considerable information has been obtained within the last five years or so on the ED_{50} values for spores of a number of species of fungi for several metal ions and organic toxicants.

All the results to date, no matter from what source, indicate a comparatively low toxicity of the fungitoxicants to fungus tissue in contrast to the high efficiency of many toxicants against other organisms. The reason for this remains obscure, but it does not seem logical to conclude that fungus spores are more difficult to destroy than other organisms. Some of the more active biocides have been discovered relatively recently, and it is quite reasonable to expect much more effective fungicides to be uncovered in the near future.

II. Metal Ions

A. UPTAKE OF COPPER BY SPORES (EARLY STUDIES, 1892–1903)

Although the discovery of the toxic action of copper to fungi is frequently credited to Prévost (68) in 1807, according to Woolman and Humphrey (80) copper sulfate was used against wheat bunt as early as 1761. The early workers were interested in the uptake of toxicants applied to the spores and published the results of many investigations along these lines. Thus, Wüthrich (81) in 1892 studied the action of copper and mercury salts on spores of a number of species of fungi and found that copper could be readily demonstrated in spores which had been suspended in dilute solutions of copper sulfate. Spores of some species took up copper more readily than others, as for example *Phytophthora infestans* and *Peronospora viticola* as compared to conidia of *Claviceps purpurea* and uredospores of *Puccinia graminis*. He also found that the prevention of germination depended on the copper taken up, for when only small amounts could be demonstrated in the spores, germination could still proceed. The spores could remove so much copper from the applied solutions that its presence could no longer be demonstrated by the chemical tests he employed.

Between 1895 and 1903 Herzberg (22), Tubeuf (75), and Hecke (21) showed that copper is not fungicidal to smut spores but merely fungistatic. Herzberg made the observation that treated spores of *Ustilago avenae*, while they failed to germinate in water, germinated readily when placed on gelatin. Similar results were obtained by Tubeuf with spores of *Tilletia tritici*. Working with this species and with spores of millet smut (*Ustilago crameri*), Hecke was able to show that when the spores are shaken in dilute solutions of copper sulfate all or almost all of the copper is removed from solution. Moreover Hecke also found that the copper held by the spores is rather firmly bound and cannot be removed by simply washing with water. When 0.5% hydrochloric acid was used, not only was the copper removed from the spores, as shown by chemical tests, but germination was again possible to the same degree as with spores never exposed to copper. Volkart (76) in 1906 confirmed these results obtained by Hecke and moreover found that treated spores would germinate in soil as well as on gelatin. This was considered an important discovery at the time since it explained why smut persisted even though seed had been treated with quantities of copper sufficient to prevent germination of the spores under the conditions of testing then employed.

Hecke was also the first worker to determine quantitatively the amounts of copper removed by smut spores when suspended in solutions of copper sulfate. He found that the spores took up the same amount of copper, about 1%, whether exposed for 5 minutes or 2 hours to concentrations of copper sulfate varying from 0.25 to 3% (10 g. of spores in 200 ml. of solution). He also noted that sulfate ions were not taken up but that nevertheless the filtrate was neutral, indicating that some alkaline spore constituents probably leached out of the spores to replace the copper removed from the solution.

Thus it had been clearly shown over 50 years ago that the action of copper on spores of the fungi causing a number of the smut diseases is fungistatic rather than fungicidal. It had also been established that at least some of the copper penetrates the spore membrane and is present in the interior of the cells. Evidence was clear that the quantities of copper removed from solution were large (as much as 1% of the weight of the spores) and that they were taken up very rapidly, within not more than 5 minutes. It was also apparent, of course, that large quantities on a spore weight basis were necessary to prevent germination and that contact must be maintained for a considerable time. Also it was shown that copper taken up is rather firmly fixed and does not pass out into water when the spores are removed from the copper solutions and placed in distilled water.

B. UPTAKE OF COPPER, MERCURY, SILVER, AND CERIUM BY CORN SMUT SPORES (1922)

Somewhat later other workers conducted experiments on the uptake of copper by fungus spores and also included other toxicants in their studies. An important contribution is that of Pichler and Wöber (67) published in 1922 in which the uptake of a number of metal ions by spores of the corn smut was determined. Experiments were carried out with copper, mercury, silver, and cerium. Quantities supplied to the spores amounted to about 24,000–68,000 μg. of the ion involved per gram of spores. They found that very large quantities, 13,000–55,000 μg. per gram, are taken up very rapidly. In most instances, nearly half of what was to be taken up was removed from solution in a minute or less. Uptake continued more gradually as the time of exposure was lengthened up to 48 hours, but most of what was taken up was removed in the first hour. Silver and mercury were taken up in larger quantities than copper. Copper was taken up more readily from copper ammonium sulfate and from a copper tartrate complex than from copper sulfate. The addition of sulfuric acid retarded the uptake of copper markedly, as did the addition of sodium chloride with respect to the uptake of mercury from mercuric chloride. The addition of small amounts of zinc sulfate to copper sulfate increased the uptake of copper. When charcoal was substituted for the spores, the uptake of copper was considerably lessened. Autoclaved spores, however, took up more copper than living spores. This greater uptake by autoclaved spores was thought to be the result of more chemical binding of the copper by spore constituents, which were more readily available after the spores were killed. The retarding effect of sulfuric acid was less with the autoclaved spores.

Pichler and Wöber concluded that some of the sulfate is also taken up by the spores from solutions of copper sulfate. This conclusion is not in accord with the results of Hecke (21) nor with those of later workers. The method used by Pichler and Wöber for determining sulfate, while not fully described, seems open to question. According to these authors the uptake does not in any respect follow the adsorption formula. Unfortunately, effects on germination were not noted in any of the tests by these authors; therefore, lethal doses were not determined.

C. QUANTITIES OF COPPER AND MERCURY REQUIRED TO INHIBIT GERMINATION OF SMUT SPORES (1927–32)

A series of studies on the uptake of copper and mercury by smut spores is presented in four significant papers published by Bodnár and coworkers (5–7,73) from 1927 to 1932. These authors confirmed results reported previously by others and in addition reported on the effects of anions on uptake of copper. They also included mercury in their studies and related effects on germination to the quantity taken up more directly than had been done previously. Copper taken up by spores of *Tilletia tritici*, from 0.125 to 2% with an average value of 1.14% of spore weight, was independent of the concentration of the applied solution. This uptake took place in 5 minutes. On longer contact, some additional uptake took place up to 24 hours. Obviously the lack of effect of concentration would be expected to apply only under conditions in which the total amount of copper available to the spores is in excess of the quantity necessary to saturate the spores. The amounts of copper taken up from solutions of the sulfate, chloride, and nitrate were similar, but when the acetate was used, quantities up to 4.28% of spore weight or 42,800 μg. per gram were taken up. Neither sulfate, chloride, nitrate, nor acetate was taken up by the spores. As far as sulfate is concerned, this is contrary to the results obtained by Pichler and Wöber (67) with corn smut but is in agreement with the conclusions of Hecke (21).

The copper taken up is not replaced by amines, as suggested by Volkart (76). The copper was found to exchange to about 55% with hydrogen ions and about 45% with ions of the alkalies, such as calcium and magnesium (5). Uptake of copper followed the Adsorption equation, in contrast to the results of Pichler and Wöber (67). Copper taken up by the spores up to 5000 μg. per gram of spores had no effect on germination. When larger amounts had been taken up, germination proceeded only after enough of the copper had been removed, either by treating with dilute acid, or as a result of leaching out of the spores into soil, to reduce the concentration of copper to below 5000 μg. per gram. When copper ammonium sulfate was used, larger amounts of copper were taken up from the same concentrations than when other forms of copper were supplied. Also the effect from copper ammonium sulfate was not reversible; this form of copper was fungicidal rather than fungistatic.

Terényi (73) found that spores of *Ustilago avenae* removed somewhat larger amounts of copper from the treating solutions than spores of *Tilletia tritici*. However, copper was less toxic to oat smut spores, and about 3% had to be taken up before germination was prevented, in contrast to the 0.5% required for wheat smut spores. When the exposure periods were 15 minutes or less, the action of the copper could be readily reversed; after longer periods reversal was more difficult to effect, in contrast to the results with wheat smut spores. With both species, copper ammonium sulfate was fungicidal.

1. *Factors Affecting the Uptake of Mercury*

In experiments with mercuric salts and spores of *Tilletia tritici*, Bodnár and Terényi (6) found that the uptake and toxic effects were much influenced by the particular mercuric salt used. Quantities of mercury supplied to the spores were as high as 500,000 μg. per gram of spores calculated as mercuric chloride. When mercuric acetate was used, the spores took up as much as 10% of their weight of mercury without a reduction in their ability to germinate. Smaller quantities of mercuric chloride and mercuric bromide prevented germination, and this effect was not reversible as it was with copper. About 35,000 μg. of mercury per gram of spore weight was required to prevent germination. The bromide was more toxic than the chloride. The spores took up some chloride and bromide ion in these tests, in contrast to those with copper in which anions were not taken up. It was concluded that these ions were not taken up as such but that molecules of mercuric chloride and bromide were taken up. The toxicity was presumed to be the result of the molecules being taken up and being dissolved in the lipoids of the spore membranes. It was concluded that the mercuric ion as such is not toxic because it is not soluble in lipoids. The addition of sodium chloride to mercuric chloride reduced the absorption of mercury and thereby the toxic effects. When mercuric cyanide was used, there was no uptake of mercury and germination remained unaffected. In recent experiments by Miller and McCallan (48) it was found that the toxic action of mercury, based on the quantity necessary to inhibit germination by 50%, was much reduced by adding bromide ions and even more so by adding iodide ions. Even after the spores had been removed from the treating solution containing the mercury, the addition of small amounts of iodide ion produced an immediate loss of germinative capacity.

Krahé (26) in 1924 suggested that of the three salts, mercuric chloride, mercuric bromide, and mercuric cyanide, the chloride is more active as a disinfectant because it dissociates less than the others. The greater efficacy of the bromide was apparently not known to him. He did point out, however, that the chloride is much more soluble in ether than the other salts and expressed the opinion that the decrease in effectiveness brought about by the addition of sodium chloride is due to the resulting decrease in the solubility of the mercury in other lipoids.

The effect of hydrogen and hydroxyl ion concentrations on the uptake of mercury compounds by, and their toxicity to, spores of *Tilletia tritici* was studied by Krauss (27) in 1930. He found that the effectiveness of mercuric chloride was increased by an increase in hydrogen ion concentration even though the quantity taken up was decreased. The uptake of organic mercury compounds was also increased by an increase in hydrogen ion concentration while their effectiveness as fungicides was decreased.

D. UPTAKE OF COPPER BY CONIDIA OF *Monolinia fructicola* AND BY OTHER SPECIES

No quantitative studies on the uptake of copper as comprehensive as those of Pichler and Wöber and of Bodnár and his coworkers seem to have been carried out since that time. However, evidence continued to accumulate, even if some of it may be considered indirect, that fungus spores require a large amount of copper for inhibition of germination, that the quantities necessary can be accumulated rapidly, and that the results apply to spores of many species of fungi and not primarily to those of the smut fungi. The later work was concerned with spores from such pathogens as *Monilinia* (*Sclerotinia*) *fructicola* which are grown in culture. McCallan (31) in 1930 studied the germination of spores of *Monilinia fructicola* in solutions of copper sulfate in which the number of spores, the concentration of copper, and the volume of the solution were varied. The copper available to the spores ranged from 900 to 730,000 μg. of copper per gram of spores. It was concluded that the toxicity is governed by the number of spores and the total amount of copper present and not by the concentration of the copper solutions. ED_{50} values therefore tend to be constant and depend on the ratio of the amount of copper to the quantity of spores. A value of 68,000 μg. of copper in the applied solution per gram of spores can be calculated from the results given.

Lin (29) was also interested in the toxicity of copper to spores of *Monilinia fructicola*. He did not determine the amount of copper taken up by the spores but does make the statement, based on external concentrations, that about 10×10^9 atoms of copper are required to inhibit the germination of one spore. This is equivalent to 1.8 μg. of copper per gram of spores and is several orders of magnitude lower than corresponding values determined both before and since the publication of his paper. It is not clear how the value of 10×10^9 was obtained. From data given it appears that a concentration of copper of about 0.01 μg. per ml. will decrease germination to 50% when a spore load of 31,000 per ml. is used. The amount of copper available per unit of spore weight would therefore be 562 μg. per gram, taking 0.575 mg. as the weight of 1×10^6 spores of *M. fructicola* (34). This value, while still lower than that obtained by others, is much higher than that represented by 10×10^9 atoms per spore. Lin apparently was unaware of much of the previous work on the uptake of copper since he does not refer to these publications.

Marsh (40) determined the uptake of copper by spores of *M. fructicola* in connection with the effect of magnesium, calcium, and potassium salts in antidoting the action of copper on these conidia. He found that the spores could take up large amounts of copper from dilute solutions; concentrations in the spores reached values up to 4000 times those of the external solutions. The spores took up from 3500 to 15,700 μg. of copper per gram of spores in 10 minutes from $10^{-4}M$ solutions of copper sulfate. The experiments were not designed to relate germination quantitatively to copper content, but from the data given the ED_{95} value can be estimated to be about 1000 μg. of copper per gram of spores.

Ciferri and Baldacci (11) reported on the effect of citric and tartaric acids on the fungicidal action of copper against conidia of *Alternaria tenuis* and *Plasmopora viticola*. For uptake experiments, because of the amount of material needed, they substituted yeast cells, which they considered comparable. The presence of citric or tartaric acid accelerated the uptake of copper from copper sulfate solutions. Thus, after 45 minutes, only 1 μg. of copper per gram of dry yeast was taken up from copper sulfate, whereas in the presence of tartaric acid or citric acid the uptake of copper was, respectively, 40 and 100 μg. per gram. After 120 minutes there was no difference in the three solutions, the uptake in each being about 120 μg. per gram. By this time most of the yeast cells were dead, and a loss of copper from the cells began, especially from those treated with citric acid and to a lesser extent those with tartaric acid. These yeast cells appeared to take up appreciably less copper than did the conidia of *M. fructicola* or the smut spores.

More recently Akai and Itoi (1) studied the uptake of copper by conidia of *Cochliobolus miyabeanus*. In agreement with earlier work they found that large amounts are taken up rapidly and that the effects of the copper can be reversed by treatment with dilute acid. From the data given it is possible to calculate an ED_{50} value of 12,000 μg. of copper per gram of spores for spores exposed for one hour to copper sulfate solutions ranging from 0.25 to 2.0%. The results obtained by these workers are in agreement with those published by early workers with smut spores. In a note by Kirby (25) it is reported that spores of *Botrytis cinerea* took up large amounts of copper very rapidly from dilute solutions of the sulfate. At low concentrations spores removed over 50% of the copper ions present. The increased copper content of the spores required to give a fungistatic ED_{95} value rather than ED_{50} was quite small.

It may be of interest to note that copper can also be accumulated by tissues of other organisms as well. Mazia and Mullins (43) immersed leaves of *Elodea canadensis* in solutions of $Cu^{64}Cl_2$ containing only 10^{-10} moles of copper per liter and found that within 30 minutes the internal concentration was 1.2×10^4 that of the applied solution; after 6 hours this was increased to 2.9×10^8. The copper was bound within the cells and could not be readily exchanged for alkali ions supplied externally, but it did exchange with nonradioactive cupric ions and with gold ions. The authors suggest that the toxic effects produced by low concentrations of copper solutions are the result of the ability of cells to accumulate large amounts against a concentration gradient.

E. PRESENCE OF TOXICANTS WITHIN SPORES AND OTHER CELLS

Many of the workers interested in the uptake of toxicants by spores refer to the process as adsorption, which of course assumes uptake only on the spore surface. However, as early as 1892 Wüthrich (81) was able to show the presence of copper in spores which had been suspended in dilute solutions of copper sulfate. Goldsworthy and Green (17,18) in 1936 and 1938 reported the results of their investigations on the uptake of copper by spores of *Monilinia fructicola* and *Glomerella cingulata* from Bordeaux mixture. By means of reagents which form colored precipitates with copper, the presence of copper inside the spores was demonstrated. The distribution of the copper was found to be uniform throughout the protoplasm. The cell walls did not appear to adsorb or fix any of the copper. Uptake of copper was related to activity of the spores. Germinating spores took up more copper than dormant spores. Mycelium, as well as spores, if killed prior to contact with soluble copper salts, readily took up the copper. Copper so taken up was easily removed by washing, while that taken up by living cells was much more firmly fixed.

The work of Mudd and Anderson (54) is of particular interest in a consideration of the distribution of metals in cells after exposure to dilute solutions. They worked with bacterial cells and used the electron microscope to study the distribution of the absorbed metal ions. Results with silver, and also lead, mercury, and nickel, with cells of *Fusobacterium*, *Eberthella typhosa*, *Shigella dysenteriae*, and *Vibrio comance* showed the inner protoplasm to be selectively darkened, but not the cell walls. Exposure to the metal salts was limited to 30 seconds. Diffusion through the cell walls and combination with the protoplasm thus took place very rapidly. More recently Harris, Eisenstark, and Dragsdorf (20) have studied the location of mercuric ions taken up by cells of *Eschcrichia coli*, *Salmonella pullorum*, and *Azotobacter agilc* and have concluded that the mercury is not deposited on the outer surface of the cells. By first saturating the cells with mercuric ions, then treating with hydrogen sulfide, they showed the presence of intracellular crystals identified by X-ray diffraction analysis as mercuric sulfide. In focusing the electron microscope most of the crystals of mercuric sulfide appeared in sharp focus at one level, indicating that they were located on a surface rather than distributed throughout the cytoplasm of the cell. It is suggested that cations are adsorbed on the cytoplasmic membrane of the cell.

Tröger (74) studied the uptake of copper by spores of *Fusarium decemcellulare*. After exposing the conidia to copper sulfate solutions he detected copper distributed in the protoplasm. Dead spores gave strong tests for Cu^{2+}, while living spores gave no tests until they were killed as by heat. The author concludes that the cells are able to reduce Cu^{2+} to Cu^{+}, which again becomes oxidized when the spores are killed. He considers the reduction of Cu^{2+} to Cu^{+} to be a detoxification mechanism. When reductase inhibitors, such as ethyl urethan, were added the copper solutions were more fungicidal. When conidia were placed in the supernatant from Bordeaux mixture preparations, they germinated freely, but germination was inhibited by contact with Bordeaux mixture particles. As the amount of copper taken into the spores was the same under both conditions, it is concluded that the fungicidal effect cannot be the result of copper taken up. The surfaces of the particles in Bordeaux mixture and of $Cu(OH)_2$ were found to have a strong oxidizing action, whereas conidia that have germinated or are capable of germination exhibit strong reducing properties. It is suggested that the lethal action of these preparations is associated with their ability to oxidize important constituents of the conidia.

F. DETERMINATION OF INNATE TOXICITY BY RADIOISOTOPE TECHNIQUES

The uptake of metal ions and the effects on germination of fungus spores have been reported in a series of papers by Miller, McCallan, and Weed (33,35,45–53). The ions investigated included silver, mercury, zinc, cadmium, copper, and cerium. The studies were carried out on spores of *Monilinia fructicola*, *Alternaria oleracea*, *Aspergillus niger*, *Neurospora sitophila*, *Venturia inaequalis*, *Venturia pyrina*, *Glomerella cingulata*, *Rhizopus nigricans*, *Myrothecium verrucaria*, and *Cephalosporium acremonium* and on cells of *Saccharomyces cerevisiae*. The radioisotopes used in most of these investigations made it possible to determine quantitatively very small amounts of the ions involved and to use more than one toxicant in an experiment and thus determine interactions between ions. Tests were also conducted with labeled spores so that the effect of various toxicants on the outward movement of cell constituents could be studied. Samples of the spores being treated were taken at appropriate intervals during the uptake studies so that effect on germination could be directly related to the quantity of ions removed from solution by the spores. By plotting the quantities taken up by the spores against the germination response on log-probability paper, straight lines were obtained from which the ED_{50} values could readily be obtained (30,78).

In conformity with the results of others it was found in general that the various toxicants studied were taken up extremely rapidly from dilute solutions. With most of the toxicants—and this applies also to organic fungicides (see Section IV)—this uptake is so rapid that the spores have already taken up a lethal dose (if the proper total quantity of toxicant in relation to the weight of spores being treated is present in the applied solution) by the time required to take a sample of the treating solution for assay. The spores can then be immediately removed from the fungicidal solution and will have lost their ability to germinate. Such a rapid inhibition does not occur, however, with copper, as found by previous workers, nor with cadmium and zinc. With these three metals a comparatively long period of contact is necessary before failure to germinate occurs when the spores are subsequently placed under conditions favorable for germination.

1. Silver and Mercury

Silver is the most toxic of the ions studied and is also more toxic than the organic fungicides investigated. ED_{50} values for the spores of five species of fungi were found to range from 90 μg. of silver per gram of spore weight for *Venturia pyrina* to 560 μg. per gram for *Aspergillis niger* (49,51). Quantities corresponding to several times the ED_{50} values are taken up in less than 30 seconds after the addition of the silver and, as will be discussed below, effects on the spore membranes also become apparent in a very short time. The evidence indicates that the lethal effect following the uptake of silver occurs almost immediately.

Not only is silver taken up rapidly by spores but it is also capable of occupying receptor sites in spores in competition with other ions added before the silver, together with the silver, or subsequently in an attempt to displace silver previously taken up (49,53). In the experiments on competition among various ions for receptor sites, silver was used at a concentration of 2160 μg. per gram of spores and the other ions were supplied at concentrations 50 times higher on an equivalent basis. When the other ions were added with the silver, the uptake of silver was retarded during the first 10 minutes by these ions in the following order of decreasing effect: mercurous, mercuric > copper > cadmium > cobalt > nickel > zinc. Zinc had no retarding effect, and mercury ions prevented all uptake during this period. On further exposure for 15 hours the quantities of silver taken up in the presence of zinc, copper, cadmium, cobalt, and nickel were not much less than in the control, while uptake was still reduced 75–85% by the mercury ions. When spores were pretreated for 1.5 hours before the addition of silver, the uptake of silver was retarded in the following order for the first 10 minutes: mercuric > mercurous > copper > zinc, cadmium, nickel. After another 19 hours the retarding effect of all the ions had been overcome except that uptake was still reduced 32–54% by ions of mercury. When the spores were first treated with silver and then the possible displacement of the silver by the subsequent addition of the other ions determined, only the ions of mercury displaced an appreciable quantity of the silver. When nonradioactive silver was added, the exchange between the two forms of silver was complete.

It is therefore apparent that only the mercurous and mercuric ions, and to a lesser extent those of copper, provide serious competition for receptor sites for silver. With time silver can replace other ions previously taken up even if these ions are or were present in concentrations 50 times higher. Moreover, spores can remove from dilute solutions 90% or more of the silver ions present. This great affinity for silver with the ability to concentrate the metal at levels several thousand-fold compared to the ambient solution no doubt accounts for the so-called oligodynamic action of silver. This term, first used by Nägeli (57), is much quoted, especially in the bacteriological literature, as if it involved some rather mysterious effect exerted by the metals at great dilutions. The distinction is sometimes made that silver nitrate at 10 p.p.m. produces ordinary or poisoning death and that at greater dilutions the effect is oligodynamic (70). Many studies have been carried out in efforts to explain such oligodynamic action. As far as fungus spores are concerned, the action of silver is not different at lower levels of concentration, and the effects produced depend merely on the dose level reached in the spores.

In view of the competition between silver and mercury for receptor sites in fungus spores, as shown when the effect of mercury on the uptake of silver is studied, it was surprising to find that, when the reverse relationship was investigated, silver increased the apparent uptake of mercury (49,53). When spores are pretreated with silver or when silver is present with mercury, the apparent uptake of mercury is much increased. Probably this results partly because silver has a marked and rapid effect on the semipermeability of the cell membranes so that cell contents leach out readily. Some of these cell contents can react with mercury and thus produce an apparent uptake. However, even small quantities of silver, which have very little effect on germination, and therefore presumably on leaching out of cell contents, can increase the uptake of mercury about 100%. This suggests that the membrane may be affected in such a way as to permit increased inward movement of materials.

The effect of silver on loss of cell constituents was studied with the use of spores labeled with P^{32}. The outward movement of phosphorus compounds began within a minute or two after the addition of a high concentration of the silver. Spores of *Monilinia fructicola, Neurospora sitophila,* and *Aspergillis niger* (46,47,49) gave similar results, except with the first species the effects were somewhat more rapid. The leaching out of the phosphorus compounds was not merely the result of the destruction of the ability of the spores to germinate, since other agents producing inhibition did not have comparable effects on cell permeability. Treatment with copper brought about some leaching out of cell phosphorus compounds, but this required a much longer time and the total quantities were usually less than with silver. Other metals, such as mercury, cadmium, and zinc, could inhibit germination without releasing cell contents.

Time and concentration studies indicate that the effect on the cell membrane takes place before germination capacity is lost. The effect on the permeability may, therefore, be the means by which silver kills the spores.

Mercury is also taken up readily by spores from dilute solutions but the quantities required to inhibit germination are much higher than for silver. ED_{50} values approximate 5000 μg. per gram of spores (53). When the mercury is supplied as mercuric acetate, the addition of bromide or iodide ions, especially the latter, greatly increases the toxicity of the mercury (48). Presumably the added ions aid the mercury in reaching effective receptor sites. Bodnár and Terényi (6) found the chloride and bromide to be more toxic than the acetate; they apparently did not test the iodide.

2. *Cadmium and Zinc*

Cadmium and zinc are much less toxic to fungus spores than silver, mercury, and copper. In studies with the labeled ions it was found that spores did not take up these ions very readily (49). Spores of *Monilinia fructicola* and *Aspergillis niger* took up much larger quantities than did those of *Neurospora sitophila*. Effects on germination were not determined solely by the quantities taken up; length of time of exposure to the ions was more important than the quantity taken up by the spores. Quantitative uptake studies were not made with copper, but with this ion, too, it was found that time of exposure was important. This would be expected in view of the fact that copper is essentially fungistatic in its effect, while the tests made in these investigations determined fungicidal action.

Considerable differences occur between spores of different species in their ability to take up various materials. Spores more active with one toxicant than those of another species may show a reverse relationship when a second toxicant is tested. Such differences could account for species specificity, but, in general, lack of ability to take up the toxicants has not been associated with specificity.

3. *Cerium*

Cerium was taken up much more readily by spores of some species than by others (51). It was found to be relatively nontoxic to the spores included in the tests, and an ED_{50} value could be obtained only for spores of *Monilinia fructicola*, namely, 4600 μg. per gram, although spores of *Aspergillus niger* and *Alternaria oleracea* took up much larger amounts under comparable conditions. No toxic effects were noted with these spores, although quantities taken up were as high as 7000–8000 μg. per gram of spores. Spores of *Neurospora sitophila*, which are active in taking up most of the toxicants studied, took up less cerium than the spores of any of the species included; under the same conditions amounts taken up were sometimes as little as one-tenth of those taken up by *Aspergillis niger*. Cerium, of course, is not important as a fungicidal agent, although it had been used in Europe some time ago as a substitute for copper (19,79). It was included in the uptake studies of Pichler and Wöber (67) and has been reported among the more toxic elements to various fungi in fungistatic tests (10,12,39).

4. Nonidentity of Receptor Sites for Some Toxicants

Studies carried out with cerium, silver, and 2-heptadecyl-2-imidazoline, in which spores of five species of fungi were exposed to various combinations of these toxicants in simultaneous and consecutive treatments, have indicated that these three toxicants do not have receptor sites in common (52). The spores took up the toxicants at about the same rate, and to about the same degree, whether the toxicants were presented to them singly, simultaneously, or consecutively. Exposures of the spores to a second or third toxicant did not result in release of toxicant previously taken up. With fungitoxicants more closely related chemically, independent uptake would not be expected to occur. An example of complete lack of independence on uptake is the interference between neodymium and cerium (45,46, 53). The spores did not distinguish between these two rare earths. Cerium taken up by spores of *Aspergillus niger* was found to exchange just as readily with neodymium as with cerium itself.

In experiments in which both cerium and sulfate were labeled it was found that little if any of the sulfate was taken up by the spores. This lack of uptake of the anion is in accord with earlier results of Hecke (21) and Bodnár *et al.* (7) in studies with copper sulfate.

III. Sulfur

Elemental sulfur is the only one of the toxicants used to control fungus diseases known not to accumulate in the spores with which it comes in contact. Spores rapidly reduce the sulfur to hydrogen sulfide, which is then released (38,41). This reduction in all likelihood takes place in the spores or at least in the spore membrane, for spore extracts or ground-up spores do not reduce sulfur to any appreciable extent. The reaction is remarkably rapid considering the extremely low solubility of sulfur in water. The hydrogen sulfide is not produced as the result of a simple reaction between sulfur and SH compounds (38), since carbon dioxide in equimolar quantities is one of the products of the reaction (36). Moreover, fungus spores in contact with elemental sulfur produce hydrogen sulfide at a more rapid rate than glutathione and sulfur mixtures under the same conditions (50).

Since sulfur has to be taken up by the spores for reduction to hydrogen sulfide to occur, the quantities of hydrogen sulfide produced can be utilized to measure sulfur uptake (50). The rate of hydrogen sulfide production in micrograms of hydrogen sulfide per gram of spores per hour for the first 2 hours for spores of nine species ranged from 6400 for *Cephalosporium acremonium* to 23 for *Rhizopus nigricans.* The tests were run in such a way that the hydrogen sulfide was removed by a stream of nitrogen as it was being given off by the spores. The results demonstrate the very high reducing power of the spores for sulfur with a production of hydrogen sulfide which is very large on a spore weight basis. This suggests that the compound involved in the reaction with sulfur has to be regenerated, since it is unlikely the spores would contain a sufficient quantity to reduce the large total amounts which are involved. In general, hydrogen sulfide production continues as long as the spores retain their capacity to germinate. The relative reactivity of the spores of the various species is not correlated with surface area. The quantities of hydrogen sulfide which can be given off before germination is reduced to 50% vary with the different species but may be more than 10,000 μg. per gram of spores.

These results suggest that hydrogen sulfide is not nearly so toxic to fungus spores as had been previously believed. It had been quite generally accepted that sulfur kills fungus spores because of the lethal effect of the hydrogen sulfide produced (28). These more recent studies have shown that hydrogen sulfide is not as toxic to spores of some species as is sulfur itself in finely divided form. The formation of hydrogen sulfide from sulfur may actually be a detoxification mechanism. When large amounts of sulfur are reduced, it is possible that the interferences with the normal hydrogenation and dehydrogenation reactions of the cells may account for some toxic effects (50).

Spores suspended in solutions containing sulfur in finely divided form can reduce all the sulfur present. With species that do not reduce sulfur actively, the sulfur remains outside the spores. This can be shown by adding spores of another species active in reducing sulfur after which all the sulfur present can be recovered. It has been suggested by Horsfall (23, p. 167) that the spores which actively respond to sulfur contain more fatty material in their membranes in which the sulfur can dissolve.

Goldsworthy (16) in 1928 studied the action of lime sulfur on uredospores of *Tranzschelia punctata.* His results indicate that the lime sulfur readily penetrates the spores, and he was able to identify particles of sulfur in the spores and germ tubes.

IV. Organic Toxicants

1. Glyodin Base

The use of organic fungicides came into practice much later than that of sulfur and the metals and therefore much less work has been carried out with them. Extensive studies have been made, however, with C^{14}-labeled 2-heptadecyl-2-imidazoline, the free base of glyodin (51). This toxicant is taken up exceedingly rapidly by fungus spores in agreement with the results obtained with the metal ions. Accumulation against a concentration gradient is probably even greater than with the metals. Spores can readily take up quantities representing a 10,000-fold accumulation. The innate toxicity of this chemical is relatively low, ED_{50} values of 9300 and 5800 μg. per gram of spores were obtained for *Venturia pyrina* and *Neurospora sitophila*, respectively. Because of the rapid rate of uptake and the degree of accumulation possible with this toxicant, control of germination can easily be attained. Spores of all species do not have the same affinity for the imidazoline. In a comparison of ten species each exposed to 2160 μg. per gram of spores, over 50% of the available toxicant was taken up in 5 minutes by spores of *Neurospora sitophila*, *Venturia pyrina*, *Monilinia fructicola*, *Venturia inaequalis*, and *Cephalosporium acremonium*. The spores of the five other species in the test took up 13–30% of the toxicant supplied. The spores taking up the smallest quantity were those of *Aspergillis niger*, which took up only about one-sixth as much as those of *Neurospora sitophila*. It will be recalled that with cerium the opposite holds: spores of *A. niger* may take up as much as 10 times more than those of *N. sitophila*.

2. Dichlone

The fungicide 2,3-dichloro-1,4-naphthoquinone (dichlone) is also taken up rapidly by fungus spores (45). This toxicant is the most effective of the organic fungicides so far studied and is only exceeded by silver among the inorganic fungicides. ED_{50} values range from 400 to about 600 μg. per gram of spore weight for four species studied. An interesting feature of the action of dichlone is that it is comparatively nontoxic to spores of *Myrothecium verrucaria* at levels which are toxic to spores of the four other species investigated. Spores which had taken up 1400 μg. per gram of spore weight germinated as well as the controls. This is an interesting example of species specificity in which uptake is not retarded but the toxicant nevertheless remains relatively ineffective.

3. Dithiocarbamates

Studies with the dithiocarbamates are complicated by the fact that these fungicides are unstable. The dithiocarbamates in solution, especially in the presence of acids or certain cell constituents, give off appreciable quantities of carbon disulfide (77). Determination of ED_{50} values for fungus spores, using S^{35}-labeled compounds, is therefore subject to rather large errors because of loss of labeled carbon disulfide. Spore constituents such as glutathione and cysteine react with the dithiocarbamates to give off carbon disulfide, and relatively small changes in acidity have a marked effect on the stability of the dithiocarbamates. Studies have been carried out by Palmer *et al.* (59,60) and by Munger *et al.* (55,56) using S^{35}-labeled compounds and an alkaline buffer to increase the stability of the compounds. The results indicated that spores of *Helminthosporium sativum* and *Monolinia fructicola* take up much larger quantities of sodium *n*-propyldithiocarbamate than of the corresponding dimethyl derivative, even though the former is less toxic on a weight basis. Although it has not been possible to determine ED_{50} values on a weight basis with much accuracy, it is clear that they are of the same order of magnitude as those found for other fungicides studied.

4. Other Organic Toxicants

Barnes (3) worked with C^{14}-labeled 8-quinolinol and the copper chelate. He did not determine ED_{50} values but found that spores of *Aspergillus niger* were much more active than the corresponding mycelium in taking up the nonchelated material. Recent studies by McCallan and Miller (35) on comparative uptake by spores and mycelial pellets of *Aspergillus niger* and *Alternaria oleracea* show that the rate is much slower for the mycelium. However, ED_{50} values for silver and dichlone were comparable for conidia and mycelium.

Burchfield and Storrs (9) have determined the uptake of 2,4-dichloro-6-anilino-*s*-triazine and the *o*-, *m*-, and *p*-chloro derivatives by spores of *Neurospora sitophila*. With the *o*-chloro derivative maximum uptake took place in 10 minutes, after which some release took place, followed by a second period of much slower uptake. Using spores labeled with P^{32} it was found that during the period when some release of the triazine took place other cell constituents as measured by P^{32} were also released. These effects were not sufficient to affect germination seriously. The quantities required to inhibit germination 50% were about 1500 μg. per gram of spores for the three chloro derivatives.

Ross and Ludwig (71) studied the uptake of a series of alkylethylenethiourea compounds including the *N*-*n*-ethyl, butyl, amyl, hexyl, heptyl, octyl, decyl, and dodecyl derivatives. Lipoid solubility and uptake increased as the length of the hydrocarbon chain increased. Assuming a value of 0.575 mg. for 1,000,000 spores of *Monilinia fructicola* (34), 5.8 mg. of spores per ml. were exposed to 20 μg. per ml. of the derivatives for 2 hours, and 120, 160, and 450 μg. per gram of spores of the hexyl, heptyl, and octyl derivatives, respectively, were taken up. With the lower members of the series no measurable uptake took place. In further experiments with the octyl derivative and higher concentrations, quantities up to 2300 μg. per gram of spores were taken up. These amounts, however, were not sufficient to prevent germination of the spores. These compounds are thus a further example of the very large quantities necessary to exert a toxic effect. When spores which had accumulated these compounds were resuspended in water, a considerable release of the chemical previously taken up took place. The authors conclude that a distribution equilibrium takes place between the spores and the external solution in accord with the Ferguson principle (2,13). In general this does not occur with the other fungicides, both organic and inorganic, which have been studied. Most of these seem to be firmly held and can only be separated from the spores by rather drastic treatments.

V. Permeability of Spores and Distribution of Toxicants within the Spores

The results of studies on uptake seem to indicate that penetration of materials into fungus spores does occur more readily than may be generally believed. It is difficult to get direct proof as to the areas in the spores where the toxicants accumulate. A number of the articles cited earlier definitely indicate penetration. Rowley and coworkers (72) disintegrated bacteria which had been exposed to penicillin and concluded that the antibiotic was not associated with the cell walls but was fixed in the interior of the cells. Miller and McCallan (49) disintegrated spores of *Neurospora sitophila* which had taken up silver and found that the silver was associated with protoplasmic contents which remained in the supernatant after fragments of cell walls had settled to the bottom. Owens and Miller (58) studied the distribution of C^{14}-labeled dichlone and glyodin base, and of Ag^{110}, Zn^{65}, Cd^{115}, Hg^{203}, and Ce^{144} in cells of *Neurospora sitophila* and *Aspergillus niger* after the conidia were disintegrated sonically. They concluded that these toxicants are absorbed by the fungus spores and not merely adsorbed on the surface.

Ready outward and inward movement of some ions was shown to occur with spores of *Asperigillus niger* when the spores were grown in the presence of Zn^{65} and replacement and exchange studies were made subsequently (49). It was found that 75% of the zinc in the spores exchanged within a few minutes with externally supplied non-radioactive zinc. It was also replaced to about the same degree with cerium added to the ambient solution. This movement of the ions in question took place under conditions in which germination remained unaffected.

A better understanding of the nature of the cell membrane would aid in clarifying concepts on toxicant uptake. In general, little is known of the composition of spore cell walls. Kaplan (24) in a recent study concluded that the basic membrane of the Ascomycetes and Basidiomycetes is generally of a chitinous nature.

VI. Interpretation of Dosage-Response Curves

The application of the dosage-response curve to the study of fungitoxicants has proved a useful tool, especially as regards comparisons of effectiveness at the same level of response, e.g., the ED_{50} or ED_{95}. In the commonly used method of Bliss (4,15) the response in probits is plotted against the logarithm of the dose; or, in simplified procedures (30,78), the data are plotted directly on logarithmic-probability paper. The use of the probit unit assumes theoretically that the individual lethal doses, or comparable responses, are normally distributed. Until the advent of radioisotope techniques the dose had been based almost exclusively on external concentrations.

On the premise of a normal distribution, Parker-Rhodes in a series of papers (61–66) has developed an elaborate hypothesis. He considers that the action of a toxicant may take place in a series of steps and that the more steps required, the greater will be the variability in the response of the spore population because the final variability will be the sum of the variabilities of all the steps. Hence, the more steps, the flatter the dosage-response curve. Linear curves are obtained by plotting the response against the concentration raised to a power α, the index of variation. The actual toxicant is considered to be the compound with the steepest slope. A more detailed discussion of this ingenious hypothesis has been given previously (32). However, a major criticism is that the slopes are based on external concentrations.

The recent availability of data on the actual uptake of fungicides by spores using isotope tracer techniques (45–47,49,51,53) has permitted a comparison of external and uptake concentrations. Preliminary studies (33) have been made in which the μg. of toxicant taken up per mg. of spores has been plotted against the logarithm of the total available μg. of toxicant per mg. of spores, i.e., the external concentration. It was found that with many curves there was a straight-line relationship which showed that the amount of toxicant taken up by the spore was proportional to the logarithm of the external concentration. However, with other curves a break and flattening appeared at the lower dosages which perhaps indicate that the mechanism of action—or, more probably, the uptake—occurs in two steps. To date most of the dosage-response curves based on uptake data have given orthodox straight lines when plotted in probit logarithmic units.

Because the slope of the dosage-response curve is a measure of the distribution of individual lethal doses, numerous investigators (e.g., 23,42,62,69,71) have attempted to assess the mechanism of fungicidal action by comparing the slopes of the dosage-response curves. However, such comparisons have been based on external concentration. If the internal dose is a function of the logarithm of the external concentration, then it must follow that only if the uptake of two toxicants is proportionally the same with increasing concentration can the slope of their internal dosage-response curves be similar. Burchfield and Storrs in a recent communication (9) and in unpublished data have shown that two isomers of triazine, which presumably have the same mode of action, are taken up by spores of *Neurospora sitophila* in amounts approximately proportional to the logarithms of their external concentrations. However, the slopes of the uptake curves differ considerably. Thus it follows again that the slopes of the dosage-response curves based on external concentration could not be the same even if they were parallel when plotted on the basis of the amount taken up. It must be concluded that, based on external concentrations, similar slopes or dissimilar slopes do not indicate, respectively, similar or dissimilar action and in fact such comparisons can be very misleading. Even the slopes of internal dosage-response curves do not necessarily indicate mechanisms of toxicity.

VII. Comparison of Efficacy of Fungicides with Other Biocides

It is apparent that the ED_{50} values reported for fungicides that have been studied are very high compared to the efficacy of other biocidal agents. Comparisons with the most effective insecticides, herbicides, and toxicants to animals have been given in previous papers (33,47) in tabular form. Fungicides are effective from 90 to over 10,000 μg. per gram of spores, while many other biocidal agents may exert their action at a microgram or less per gram of tissue acted upon. The reviewers have not attempted to cover the literature on bactericides, as expressed on the basis of the weight of the bacteria acted upon. However, working with penicillin, Rowley *et al.* (72) have shown that 1–4 μg. per gram of bacterial weight will prevent the growth of a number of species of bacteria. Few and Schulman (14) have recently studied the uptake of polymyxin by bacteria, and while they did not relate the quantities taken up directly with toxic effects, their data indicate that ED_{50} values would probably considerably exceed those reported for penicillin.

As recognition of the necessity of determining the true toxicity of fungicidal materials increases, and emphasis is placed on developing chemicals with a high activity on a spore weight basis, fungitoxicants of greater value should be uncovered. The large gap between the activities of fungicides and of other biocides should therefore gradually be closed.

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RESEARCH ADVANCES IN SEED AND SOIL TREATMENT WITH SYSTEMIC AND NONSYSTEMIC INSECTICIDES

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CONTENTS

I. Introduction 135
II. Seed Treatment with Nonsystemic Insecticides 136
    A. Insects Susceptible to Seed Treatment 137
    B. Effective Insecticides 140
    C. Phytotoxicity and Other Seed Treatment Problems 143
    D. Methods of Treating Seed 146
III. Application of Systemic Insecticides at Time of Planting 147
    A. Physiological Aspects and Metabolic Fate within the Plant 150
    B. Seed Treatment 158
    C. Granular Application at Time of Planting 168
    D. Liquid Application at Time of Planting 169
IV. Application of Systemic Insecticides to the Soil after Planting 170
V. Residue Problems 171

References 178

I. Introduction

Pesticides applied on or into the soil have attained a wide acceptance among farmers and scientists alike for plant protection from attack by a wide variety of noxious pests affecting agricultural production. It is only in recent years, however, that the use of soil insecticides has become a widely recommended practice in the field of entomology. The development of the synthetic organic insecticides, beginning with DDT, provided the necessary means to successfully combat many highly destructive, phytophagous insects which previously were relatively safe from available chemical controls. A long residual period of activity, low phytotoxicity, and ability to be toxic by contact in low dosages are some of the basic requirements of a successful soil insecticide which many chlorinated hydrocarbon insecticides provide.

In recent years the development of the organophosphorus systemic compounds has provided new and hitherto unexplored ramifications in the development of soil insecticides. Commonly a soil insecticide is understood to be a chemical applied on or into the soil for control of destructive insects which spend part or all of their life history in or on the surface of the soil. That systemic insecticides applied to the soil do not completely fit this concept of a soil insecticide is evidenced by the fact that preliminary research indicates the primary usefulness of these compounds to be for control of pests inhabiting the aerial portions of the plant. Actually, results to date with the currently available systemic insecticides cast some doubt upon their value for control of most soil-inhabiting insects.

The subject of soil insecticides has reached such enormous volume that it is impossible to cover the field in detail in one short review. For a broad coverage of this subject the reader is referred to the excellent review of Lilly (88). To permit detailed discussion, conventional insecticides are considered only in terms of seed treatment. The full import of the systemic insecticides cannot be realized by discussing seed treatment alone, however; therefore, for this group the various other methods of application at time of planting are also considered.

II. Seed Treatment with Nonsystemic Insecticides

The use of chemicals on seeds to kill or repel insects living in the soil is certainly not a new development. Thomas (134) in an extensive review of the literature cited a large number of papers dealing with early attempts to control wireworms by treating seed. Most early attempts were with stomach poisons or repellents. Compounds such as strychnine (93), mercuric chloride (93), Paris green (93,94), sodium arsenite (93), lead arsenate (94), coal tars (122), and others were tried. It was pointed out that none gave satisfactory results (134). Thomas concluded from work by King *et al.* (75) that the stomach poisons were ineffective at least partially because wireworms frequently discard the first few bites from a seed and do not actually feed until they reach the softer tissue underneath the surface. Later, Woodworth (139) reported on this feeding habit as well and came to similar conclusions. It is small wonder, then, that successful seed treatments were not discovered until the development of the highly effective chlorinated hydrocarbon insecticides which have the ability to kill by contact.

A. INSECTS SUSCEPTIBLE TO SEED TREATMENT

As seed treatments affect only a limited soil area, only the phytophagous soil-inhabiting insects which attack or come into close proximity to the seed are susceptible to seed treatment. Lange *et al.* (83) found, however, that old seed parts of spinach and sugar beets remaining in the soil which had been treated with benzene hexachloride retained partial effectiveness in killing wireworms for several months. They further reasoned that seed treatment was not a permanent control measure but was a method of treatment for obtaining temporary control and a good stand of healthy plants. For this reason it must be repeated for each crop.

1. Seed-corn maggot, *Hylemya cilicrura* (Rond.). Life history studies have shown the reasons for this pest being particularly susceptible to seed treatment. Reid (110) reported that the adult deposits her eggs on or near a source of larval food. Freshly cultivated fields are attractive to the flies. Upon hatching from the eggs the tiny larvae work through the soil in search of food. It was found that living and decaying organic matter were food sources, and that sprouting seed and tender seedlings were quite attractive to the larvae. Reid (109) in an earlier paper reported that organic fertilizer such as cottonseed meal, fish meal, etc., attracted the flies and stimulated oviposition, which is the reason so many research workers have scattered moistened fish meal over their plots in an attempt to assure themselves of a maggot population in their experiments. Lange *et al.* (82) stated that damage from seed-corn maggot in California is more severe during wet, cool periods than under conditions when germination and growth occur rapidly. Double-cropping practices intensified the problem by providing large amounts of organic matter to attract the flies (38,82). For example, Elmore (38) reported that effective chemical control of wireworms in southern California permitted planting of winter crops previously avoided to discourage wireworms, and these crops added decaying plant matter to the soil which aggravated the problem of the seed-corn maggot in subsequent spring plantings.

2. Onion maggot, *Hylemya antiqua* (Meig.); cabbage maggot, *H. brassicae* (Bouché); and turnip maggot, *H. floralis* (Fall.). In general, seed treatment methods have not been considered very promising for control of this group of root maggots. In contrast to the adults of seed-corn maggot, which are attracted to decaying organic matter and frequently lay their eggs upon freshly cultivated soil, the adults of these maggots lay their eggs primarily upon the young plants or on nearby soil. Consequently, larvae of the onion, cabbage, and turnip maggots have less opportunity than those of the seed-corn maggot to come into contact with treated seed or old seed parts. A method of application to be most effective, therefore, must maintain adequate amounts of insecticide near the roots (76). Nevertheless, promising results have been reported using seed treatment for control of onion maggot (86).

3. Carrot rust fly, *Psila rosae* (F.). This species deposits eggs about the base of plants. Upon hatching the larvae work down into the soil and attack the tender tips of the roots. It has been reported that seed treatment is promising for control of this pest (86,140) and is practiced commercially in Great Britain (5,6,62).

4. Southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber. The adults of this species lay their eggs in the soil around the host plants. The larvae feed on sprouting seed and tunnel into the roots of both younger and older plants. In addition, they bore into the stalk above roots and may kill the bud on small plants by feeding on the crown. Seed treatment has been listed as somewhat promising in control of the larvae (7,36).

5. Seed Collembola, *Onychiurus sp.* Collembola occasionally feed on the tender parts of germinating seed. On seedling bean plants they may attack the roots, causing stunted and delayed growth, and also may feed on plumules so that they later appear ragged. Limited tests with seed treatment on beans indicate control may be possible (85).

6. Seed-attacking beetles, *Agonoderus comma* Fabr. and *Clivina impressifrons* LeConte. Although these beetles are listed as being primarily predaceous, the adults sometimes attack germinating seed. In laboratory tests it was demonstrated that they are susceptible to seed treatment (129).

7. False wireworms, *Eleodes spp.* Larvae of this group of insects, when abundant, can cause considerable injury to germinating seed. In the High Plains area of Texas it was shown that treating the seed of grain and sorghum gave good protection (27).

8. Wireworms. As is the case with seed-corn maggot, there is a great amount of international interest and research on control of wireworms with seed treatment. It has been demonstrated that, for seed treatment to be most effective on wireworms, the chemical involved must seriously affect or kill within a short time (83). According to Lange *et al.* (83) the efficacy of seed treatment depends on several factors; among these the species of wireworm involved, activity when seed is planted, proportion of population attracted to seed, type of seed, and time of planting are all considered important. These workers found that 50–70% of the larger worms are normally attracted to the seed at time of germination, and usually 70–95% of these were killed. They found that this permitted a stand to become established, and even though other wireworms may attack the small plants later, the plant stand is not seriously affected. Some workers have reported disappointing results with seed treatment under conditions of heavy wireworm populations (81,88).

Many species of wireworms have been involved in seed treatment experiments. Lange *et al.* (83), in determining differences in susceptibility to benzene hexachloride, found *Aeolus sp.* to be the most susceptible species, followed by *Limonius canus* Lec., *L. californicus* (Mann.), and *Anchastus sp.* was the least susceptible. Some of the other species reported to be susceptible are *Melanotus communis* (Gyll.) (34,81), *Dalopius pallidus* Brown (34), *Agriotes mancus* (Say) (34), *Aeolus mellilus* (Say) (34), *Limonius clypeatus* Mots. (83), *Melanotus oregonesis* (Lec.) (83), and *Ctenicera aeripennis destructor* (Brown) (13).

9. Flea beetles, *Phyllotreta spp.* Control of the adult beetles on seedling plants is practiced commercially in Great Britain by treating seed of cruciferous crops with lindane. Heavy dosages are used in seed dressing and control is obtained through utilizing the systemic nature of the compound (5,6,62).

10. Seed-borne nematode. Cralley (25,26) was able to reduce whitetip of rice through controlling a seed-borne nematode by seed treatment with parathion, Systox,® malathion, and a nematocide.

11. Wheat bulb fly, *Leptohylemyia coarctata* Fall. Early attempts to control this pest with seed treatment gave conflicting but usually negative results. Subsequently it was found that the period between the date of planting treated seed and hatching of wheat bulb fly eggs was an important factor in successful control. Thus it was found that seed treatments afforded little protection in the case of early-planted wheat, but a marked and significant reduction in damage occurred in the case of late-planted seed when the date of planting was close to the time of egg hatch (48).

B. EFFECTIVE INSECTICIDES

For the sake of brevity, only those materials which have shown particular promise for use as seed treatments will be discussed. Some workers have reported chlordane as promising, but interest in its possible use waned following the development of aldrin, heptachlor, and dieldrin. Obviously, it is not possible to discuss all the dosages of the various promising insecticides described in the literature. Dosages which are effective are known to vary in different areas with both insecticides and fungicides. Consequently, to give the reader some idea of effective dosages and seed tolerance on different crops, the recommended materials and dosages from the 1957 University of California recommendations on California vegetable and field crops (135a) are shown in Table I. The predominance of lindane in this table is primarily due to the greater experience with this chemical; as time permits further experimentation with aldrin, heptachlor, dieldrin, and perhaps endrin, a wider range of recommended usage can be expected for these compounds.

Much work has been done with technical benzene hexachloride, but because of phytotoxicity problems and possible off-flavor hazards involved with its use it has received little commercial application. Sugar beet seed apparently are quite tolerant to BHC, and considerable amounts were used in California prior to the appearance of lindane (gamma isomer of benzene hexachloride) on the market (83). This insecticide is effective against the same pests as lindane.

Lindane is essentially pure gamma isomer of benzene hexachloride. Despite the development of other effective chemicals, lindane remains one of the best materials for control of insects susceptible to seed treatment. It has a rapid effect on wireworms, which is desirable, and is adequate for control of seed-corn maggot (85). A number of research workers have reported good results on control of wireworms with lindane (34,54,70,71,81,83). It has been suggested that lindane in addition to killing wireworms has some repellent effect (34,83,129). Lindane is widely recommended and used for control of seed-corn maggot. Most of the large number of papers that have appeared have reported good to excellent results (38,42,61,64,82,84, 85,105,128,129). Lindane seed treatments have also been listed as promising for control of false wireworms (27), southern corn rootworm (36), and seed-attacking beetles (129). Successful protection from attack by larvae of the wheat bulb fly was obtained when wheat seed treated with γ-BHC was planted at a date close to the time of egg hatch. Gough and Woods (48) found that γ-BHC prevented entrance of larvae into the stem, whereas the action of dieldrin and aldrin was somewhat delayed and killed the larvae after entry. Control of carrot rust fly larvae with γ-BHC was reported in Great Britain (140), and it is now used commercially in that country; the dosage rates (1–1.5 ounces of 75% lindane per pound of seed) are much higher than those employed in the United States for control of other soil pests (5,6,62). Utilization of the systemic nature of lindane for control of flea beetles is discussed in Section III.B.

For the most part, results with aldrin, heptachlor, and dieldrin have been quite similar. Lange *et al.* (85) reported that all three were about equal in protecting seeds against insect attack. All gave excellent control of seed-corn maggot, but in some tests heptachlor gave a slightly quicker kill of wireworms. Kulash (81) and others have also reported all three to be effective in killing wireworms. As in the case of lindane, a number of papers have reported one or more of these materials as promising for control of seed-corn maggots (38,42,61,64,82,85,128). Lange *et al.* (84) stated that all three appeared to be slightly superior to lindane for maggot control. In addition, aldrin has been listed as promising on false wireworm (27), carrot rust fly (86), wheat bulb fly (48), and onion maggot (86); heptachlor and dieldrin on seed-attacking beetles (129), wheat bulb fly (48), and false wireworm (27). In all probability aldrin, dieldrin, and heptachlor have about the same range of effectiveness on all of these pests if tested in a similar manner.

Endrin and isodrin have shown promise as seed treatments, but only endrin is available commercially. When used at the rate of two-thirds of an ounce of the 75% wettable powder per 100 pounds of lima bean seed, these insecticides were promising for control of Collembola and appeared to be the only materials capable of controlling this pest (85). At this dosage endrin, particularly, was promising for control of wireworm and seed-corn maggot (85).

Several organophosphorus insecticides have been used in laboratory and field experiments with promising results. Lange *et al.* (83) reported parathion as promising for wireworm control on baby lima beans at a dosage of 0.5–1.0 pound of the 75% wettable powder per 100 pounds of seed. Kulash (81) at about the same dosage found it promising for wireworm control in laboratory tests but

TABLE I

Suggested Dosages of Insecticides and Fungicides for Use on Vegetable and Field. Crop Seeds (135a)[a]

Seed	Lindane, 75%	Dieldrin, aldrin, endrin, or heptachlor, 75%	Suggested fungicide for use with insecticides[c]
	ounces/100 lb. of seed[b]		
Alfalfa, clover, and other small-seeded legumes	0.66	—	Thiram, Dichlone
Barley	1.33	1.00–2.00	Ceresan M or other organic mercuries
Beans (all except large lima)	0.66	0.66	Thiram, Chloranil, Captan
Beans (large lima)	0.33	0.66	Thiram, Chloranil, Captan
Beets, chard	5.33	5.33	Dichlone, Captan, Thiram
Broccoli, cabbage, turnip, other crucifers	0.66	0.66	Thiram
Carrots	1.33	?	Thiram, Chloranil
Cantaloupes, cucumbers, watermelons, squash	1.33	0.66	Thiram, Dichlone, Captan
Corn	1.33	0.33–0.66	Thiram, Chloranil, Dichlone, Captan
Cotton	2.66	1.00–2.00?	Ceresan M, Captan, Panogen
Flax	—	—	Ceresan M
Grasses (sudan, blue, etc.)	1.33	0.66	Thiram, Chloranil, Captan
Lettuce	1.33	0.66?	Thiram
Oats, rye	0.66	0.66	Ceresan M or other organic mercuries
Okra	1.33	?	Chloranil, Thiram
Onion	1.33	2.00	Thiram
Peas	1.33	0.66	Dichlone, Thiram, Chloranil
Pepper	1.33	1.33?	Thiram
Rice	—	—	Thiram, Dichlone
Safflower, sunflower	0.66	0.66	—
Soybean	0.66	0.66	Chloranil, Thiram, Captan
Sorghum	0.66	0.66	Thiram, Ceresan M, Dichlone
Spinach	1.33	2.00	Dichlone, Captan, Thiram
Tomato	2.66	2.00	Ceresan M, Thiram
Wheat	0.33–0.66	0.66	Ceresan M or other organic mercuries

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[a] Commercial preparations containing insecticide and fungicide combinations are available and should be used according to the manufacturer's recommenda-

not as good in the field. At higher dosages Dogger and Lilly (34) found it highly toxic in both laboratory and field tests and indicated that parathion, unlike some chlorinated hydrocarbon insecticides, had no repellent effect on wireworms.

Diazinon® was found to be as effective in killing wireworms as heptachlor, dieldrin, and lindane but failed satisfactorily to prevent damage to the seed, possibly because of no repellent action (129). Starks and Lilly (128) found this material to be promising for control of seed-corn maggot.

In preliminary tests, ethyl *p*-nitrophenyl phenylphosphonothionate (EPN) gave excellent results for control of seed-corn maggot and wireworms (84).

C. PHYTOTOXICITY AND OTHER SEED TREATMENT PROBLEMS

Much of the early work with chlorinated hydrocarbon insecticide seed treatments consisted of applying the chemical without adding a fungicide. Often it was found that insecticide alone reduced the germination of the seed below that of untreated seed (60,64,84,105). The insecticides, though necessary to reduce soil insect damage, predispose the seed to greater vulnerability to soil organisms (primarily *Pythium ultimum* in California) than would normally be the case (42,61,84,85). Incorporating a fungicide with the seed treatment insecticide tends to lessen this effect.

Any treatment which places the chemical involved in as intimate contact with the seed and tender germinating plant parts as seed treatment may cause plant injury. Many early attempts in treating seed with crude or technical benzene hexachloride resulted in impaired germination, and, consequently, discouraging reports appeared in the literature. McLeod (95) noticed that pelleting seed with benzene hexachloride prevented growth of seedlings. He noted

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tions. The full compatibilities of all insecticide and fungicide combinations have not been fully determined.

[b] Slurry treatment is suggested for most seeds, but certain seeds, such as beets and spinach, cannot be slurried; these should be treated in a spray treater or the insecticides applied as dusts.

[c] For further information contact the Department of Plant Pathology, Davis or Berkeley, or follow the manufacturer's suggestions for rates; Thiram is commonly sold as Arasan, Chloranil as Spergon, and Dichlone as Phygon.

also a thickening of the shoot and a necrosis on the rootlets. Brooks and Anderson (19) using 0.5% gamma isomer benzene hexachloride at a rate of 12 pounds per bushel reported a reduction in germination of two varieties of lima beans and one variety of snap beans and sweet corn but no reduction in the cases of Black Valentine snap beans and Aristagold sweet corn. Lange *et al.* (83) found sugar beet seed quite tolerant to this material. Ristich and Schwardt (120) did not report encouraging results. Sprouting was inhibited when potato seed pieces were treated with benzene hexachloride (13). Kostoff (77,78) in a detailed study of the effects of benzene hexachloride on plants reported upon the induction of atypical growth. He found root growth suppression as well as a suppression of stem growth. Abnormal mitosis was induced in which the cells grow (resulting in polyploidy) instead of multiplying, with a resultant thickening or swelling, particularly at the growing tips of the stems and roots. This undoubtedly is the cause of the thickening of roots and stems reported in the literature (34,95,129). It is known that the gamma isomer (lindane) is the only important portion of technical benzene hexachloride insofar as insecticidal activity is concerned (83). Hocking (58) presented evidence that the gamma isomer is not the primary cause of plant deformation with benzene hexachloride. A mixture of trichlorobenzenes resulting largely from the breakdown of the alpha isomer of benzene hexachloride possesses the ability to cause plant deformation to a large degree. He found that low doses inhibit germination.

Preparations of the gamma isomer of benzene hexachloride, or lindane, became available in 1948. It was found that seed of most crops tested were tolerant to this material, and a greatly expanded seed treatment program became possible. Tolerances of different seeds to lindane were found to vary widely. If lindane is used at dosages greater than those found to be safe (see Table I for levels considered safe in California), symptoms of injury such as delay in germination, slight stunting of plants, nonabsorption of cotyledons in the case of certain bean varieties, and reduction in seedling weight may occur (83). A number of papers have noted these phenomena with lindane (34,38,70,128,129). Occasional failures with lindane have been reported in hot, dry areas (82) and in dry, sandy soil low in organic matter (61). Starks and Lilly (129) reported that corn seedlings made up initial deficiencies in green weight caused by high dosages of lindane within 18 days after planting. Jameson *et al.* (70) made similar observations in the case of grain.

Effects on germination and on seedling plants have not been as noticeable with aldrin, heptachlor, or dieldrin as with lindane (38), although several papers have noted a slight delay in germination.

There has been some question concerning storage of seed for periods of time after insecticide-fungicide coatings have been applied. Several research workers have made notes on this problem, but Hofmaster and Nugent (61) made a detailed study. The following is taken from their conclusions. Short storage periods of 3 or 4 months in commercial seed storage had no detrimental effects on treated seed either in regard to seed injury or subsequent control of seed-corn maggots or seed decay organisms provided storage temperatures were not extremely high. Storage periods of a year's duration showed that dieldrin was the only material to retain insecticidal activity at a high level. High temperatures, especially when associated with high moisture content in the seed, tended to reduce vigor of most seed; at 100°F. lima bean seed lost considerable vigor in 3 months. Lindane, aldrin, and heptachlor lost some of their effectiveness in this time at this temperature. On sugar beet seed treated with lindane and phygon there were no adverse germination effects after a 2-year storage period, but the lindane lost 50% of its insecticidal efficiency in one year (84). Lange *et al.* (83) reported storage up to 3 months under conditions not adverse to seed viability was a safe period. Jameson *et al.* (70) found no loss in seed viability and little loss in effectiveness in grain seed planted one year after treatment with lindane.

There are indications that seed of low viability (60–70% or below) should not be treated (82,128). Damaging seed in the harvesting process has been shown to be a factor in failures. Beans are particularly susceptible to mechanical injury. The treating process may injure such seed and, in addition, maggots are able to enter cracks without exposure to insecticide (82). Some seed-treating devices, particularly slurry treaters, may injure seed if not operated properly (82).

Of considerable importance in the case of certain legume seed is the finding of Starks and Lilly (128) that heptachlor, toxaphene, lindane, and dieldrin are compatible with the bacterial inoculant on soybean seed. Presumably, these were the only insecticides tested.

There are few reports in the literature on residue or "off-flavor" problems resulting from seed treatment. Treatments containing aldrin, dieldrin, heptachlor, or lindane do not create a soil residue or "off-flavor" problem because of the small amount of toxicant used per acre, usually one-fourth of an ounce or less (61). Flavor difference tests have shown that treatment of lima beans with 1.33 ounces of 75% lindane per 100 pounds of seed did not adversely affect the quality of the crop (84).

D. METHODS OF TREATING SEED

There are four methods of applying insecticide-fungicide treatments to seed (84). These are:

1. The dust method, in which measured amounts of insecticide and fungicide are applied dry to the seed in a rotating barrel, cement mixer, or other acceptable type of mixer.

2. The liquid-fixation method where, after the insecticide and fungicide are applied to the seed, $^1/_8$–2 quarts of water is sprayed on each 100 pounds of seed.

3. The spray method, in which liquid insecticides and fungicides are sprayed onto the seed while it is tumbling or mixing.

4. The slurry method, in which a thick suspension of insecticide and fungicide is applied to the seed by means of a slurry treater.

The methods in which liquid is used are preferred to the dust method because of increased efficiency and greater adherence and uniformity of toxicant distribution over the seed (83). Starks and Lilly (129) found slurries gave less injury to roots than dry applications. They reasoned that this was the result of even distribution, whereas in the dry method high concentrations may accumulate in a sensitive region on the seed. These authors found that acetone solutions offered little promise because of reduced germination. Hofmaster and Nugent (61) found dry applications inferior to the slurry method and reported the liquid-fixation method, where water without methyl cellulose was used to fix the dust, inferior to the slurry method.

In California, slurry treatments are accepted except for rough-coated seeds such as beets. Sprays are commonly applied to cereals, beets, spinach, and similar seeds.

In all applications involving liquid (slurry, liquid-fixation, and spray methods), methyl cellulose is dissolved in the water to act as a sticker. This is usually added to the water to make a 2.5–3% solution. In Great Britain linseed oil or paraffin is often used to stick the material to the seed (5,6).

It is preferable to apply the insecticide-fungicide in combination rather than separately. At the present time there are proprietary combinations of insecticides and fungicides on the market, although in some cases these do not permit the correct dosage of material for all types of seed. In many cases it is now possible to purchase seed previously treated by seed companies.

III. Application of Systemic Insecticides at Time of Planting

An enormous amount of interest is evident among research workers concerning the practicability of applying systemic insecticides to the soil during and after planting. Although a great deal of experimental work is currently being conducted, considering the potential of this method of treatment, relatively few published reports have appeared to date. In the next few years a veritable flood of papers undoubtedly will appear in the literature.

Ripper (117) pointed out that the ability of plants to translocate systemic insecticides from the root has been exploited for control methods because (*a*) a longer persistence of the systemic effect is obtained than with other methods of applying systemic insecticides; (*b*) certain systemic insecticides offer ecological selectivity while foliar applications leave them nonselective (119); (*c*) it is possible to treat plants before they have a lot of leaf, at a time when the surfaces which could absorb the systemic are confined to the stem (39); (*d*) acute toxic hazards during application are reduced as the atomization of the liquid is avoided (127). Reynolds *et al.* (114), in discussing advantages of soil applications of systemic insecticides over methods used in the past to obtain successful insect control on seedling plants, pointed out that (*a*) seedling plants are particularly susceptible to pest attack; (*b*) there is little plant surface to receive and retain insecticide deposits derived from foliar applications; (*c*) the rapid growth rate of small plants makes it difficult to obtain much more than initial mortalities with conventional insecticides, particularly under conditions of heavy insect migrations. On the basis of the results of recent research with systemic insecticides, there can be little doubt that many of these advantages can be utilized with certain systemic materials because, over a period of time, they can actively translocate from the roots into new growth in toxic amounts.

Applications made at time of planting are, in general, preventative, or so-called "insurance," applications. As such, their ultimate value should be evaluated carefully before their widespread use is recommended. There are several reasons for proceeding with caution. The economics must be considered; in most cases insecticides must pay their way by showing a profit to the farmer. On a majority of crops it is not normally necessary to make insecticide applications in the seedling stages of growth. On virtually all crops there is a complex of insects or spider mites at subeconomic population levels on the small plants, but if their feeding or presence does not result in economic damage, it is not considered a wise practice to apply insecticide just for the sake of the plants' appearance or on the chance that they would necessitate insecticide applications at a later date anyway. In cases such as these, it seems advisable to reserve applications until the pest in question becomes a problem; then, if the species is susceptible, side dressing granular formulations of systemic insecticides or some other effective manner of application would seem to be a preferable procedure. Some crops, however, almost invariably require insecticide applications in the early stages of growth. One such case might well be that of cruciferous crops, for cabbage aphid, *Brevicoryne brassicae* (L.), populations develop in most areas on the early seedling stages. Another example is alfalfa, as it has been pointed out that planting alfalfa when the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), is abundant in the area usually necessitates one or more applications of insecticide and under heavy migration perhaps the plant stand may be lost regardless (114). Another reason for caution in utilizing applications at planting time is that needless exposure of the pest complex to insecticide may hasten insect or spider mite resistance.

There is no question that systemic insecticides applied to the soil are quite selective in their action and leave biological control agencies virtually intact. Ripper *et al.* (116,119) pointed out the possibility that after treatment with a selective insecticide, resistant strains of the pest population, which survive treatment, will be destroyed by beneficial insects (presumably resident) and, thus, the segregation of strains of pests resistant to the insecticides will not occur. There is little doubt that this feature of selective insecticides is or could become a great benefit in agricultural entomology. On the other hand, the searching capacity of beneficial insects is usually not such that they can find and destroy the entire residual pest population. The end result more likely would be, therefore, a delay, possibly for an indefinite period, rather than prevention of resistance. Perhaps of almost equal value is a second possible result of using selective systemic insecticides, namely, restoration of a favorable beneficial-destructive insect balance. This would reduce the number of chemical treatments needed and lessen the exposure of the pest or pest complex in question, thereby delaying further the development of resistance. Exploring this theory further, it seems entirely possible that selectivity, through selective insecticide toxicity or through selective methods of application (soil treatments, for example), would affect only certain species in a given insect or spider mite fauna. Consequently, for all practical purposes, nonsusceptible phytophagous species are not exposed to the insecticide, which may have profound ramifications in development of resistance. To illustrate by example, in southern California the serious incursion of the spotted alfalfa aphid has necessitated repeated applications of nonselective phosphorous-containing insecticides. Not only has the spotted alfalfa aphid developed a low level of resistance in a few localities (131), but a spider mite, *Tetranychus atlanticus* McG., also developed resistance to phosphorous-containing compounds (4). Although this spider mite species is normally at subeconomic population levels in alfalfa, it became a serious problem, both in the alfalfa and in adjoining cotton fields, and it soon became apparent that Systox was no longer effective, even though this was not the compound used in the alfalfa fields. The need for a selective insecticide is readily apparent in this case, not only to spare the beneficial insect population, but to mitigate exposure to other pest species as well.

In the case of seedling plants in contrast to established plantings, however, an entirely different situation exists. Normally, there is no resident population of beneficial insects in seedling fields, and consequently they must migrate in from areas outside the confines of the field in question. If the host density is extremely low, there is little likelihood that the beneficial insect migrants will remain within the field and reproduce. This has ramifications beyond the possible survival of resistant strains, since pests establishing in the field at a later date, which may be potentially far more serious than the seedling pests, may find biotic conditions more favorable to their survival and reproduction. The possibility would appear to be favorable, therefore, of their reaching damaging population levels at an earlier date than might be the case normally.

There are a number of papers which have reviewed much of the literature available on systemic insecticides at the time of their publication. The most important ones are by Greenslade (49), Ivy (67), Jeppson (72), Metcalf (96,97), Reynolds (111), Unterstenhöfer (137), and von Rümker (138). The recent classical review by Ripper (117) in *Advances in Pest Control Research,* Volume I, contains considerable information on soil application with systemic insecticides.

A. PHYSIOLOGICAL ASPECTS AND METABOLIC FATE WITHIN THE PLANT

In 1952 a threefold method of classifying systemic insecticides was proposed based upon the fate of the compounds within the plant (115). The proposed classification was as follows:

1. Stable systemic insecticides which are not metabolized by the plant.

2. Endolytic systemic insecticides in which the toxic compound is present to 98% in its original form when ingested by the insect until it is finally decomposed by the plant.

3. Endometatoxic systemic insecticides which are transformed in the plant partially or wholly into other toxic substances, which also act as insecticides when ingested by the pest, until they are rendered nontoxic by the plant.

Stable systemic insecticides are not considered promising for use on food crops because of high mammalian toxicity and residue persistence. In general, the better-known stable systemics are selenium compounds (65,104), several derivatives of 2-fluoroethyl alcohol (123), and sodium fluoroacetate (30).

Endolytic systemic insecticides are used in agricultural production to a considerable extent. The best known of this group is schradan. It has been shown in the case of schradan that relatively small amounts of active metabolite are formed in the plant, but when ingested by the insect, the parent chemical is metabolized to other compounds including an active metabolite which is responsible for toxicity (21,22,33,35,106). Ripper (117) proposed the term *zoometatoxic* for systemic insecticides which derive their toxic action from an enzymic metabolization by the insect to an active cholinesterase inhibitor. Another compound which probably belongs to the endolytic group is Phosdrin® (OS-2046), or *O,O*-dimethyl *O*-2-carbomethoxy-1-methylvinyl phosphate. This compound has a very short period of persistence, not over 24 hours, insofar as toxicity to insects is concerned (132). According to Metcalf (97) the properties for systemic action in a chemical appear to be (*a*) sufficient water solubility to enable the compound to move within the plant sap; (*b*) ability to penetrate through roots, stems, and leaves; and (*c*) sufficient stability within the plant for the compound to exert some degree of residual toxic action. Phosdrin, then, fulfills the first two requirements, but the third is in some doubt because its primary activity in the field seems to be due to vapors and contact (132). Its usefulness as a systemic applied to the soil would appear limited.

Currently, the greatest interest among entomologists is in the group of systemic materials possessing endometatoxic characteristics. This would include both isomers which compose technical Systox®, *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate (thiono isomer) and *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate (thiol isomer). Heath *et al.* (55) found that virtually none of either isomer was found in the plant a few days after treatment. This was confirmed and the various metabolic products within the plant were identified in a series of classical papers (43,44,90–92,99,100). It was found that the metabolic process was primarily oxidative in nature and that there were seven possible products of the thiono and thiol isomers. Fukuto *et al.* (43) listed them as follows: *O,O*-diethyl *O*-ethyl-2-sulfinylethyl phosphorothionate (thionophosphate sulfoxide); *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate (thiolphosphate sulfoxide); *O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphate (phosphate); *O,O*-diethyl *O*-ethyl-2-sulfinylethyl phosphate (phosphate sulfoxide); *O,O*-diethyl *O*-ethyl-2-sulfonylethyl phosphate (phosphate sulfone); *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate (thiolphosphate sulfone); and *O,O*-diethyl *O*-ethyl-2-sulfonylethyl phosphorothionate (thionophosphate sulfone). They further found that the sulfoxide and sulfone derivatives of both isomers were the metabolic products primarily toxic to insects.

Other endometatoxic systemic insecticides currently of great interest to entomologists from toxicological and physiological as well as field-use aspects are methyl Systox (Meta-Systox®), methyl Systox thiol isomer (methyl isosystox), Systox thiol isomer (isosystox), Thimet® (3911), and Di-Syston (19639). In general, the metabolism of these compounds within the plant occurs in a fashion quite analogous to that just described for Systox, and it is primarily oxidative in nature.

Metcalf *et al.* (98) found that following uptake of Di-Syston by cotton, lemon, bean, or alfalfa plants, the mercapto sulfur is oxidized very rapidly to produce the sulfoxide, *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorodithioate, and more slowly to produce the corresponding sulfone, *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorodithioate. Both the sulfoxide and sulfone are also oxidized at the thiono sulfur to produce *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate and *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate. It was found by the above workers (98) and by Bowman and

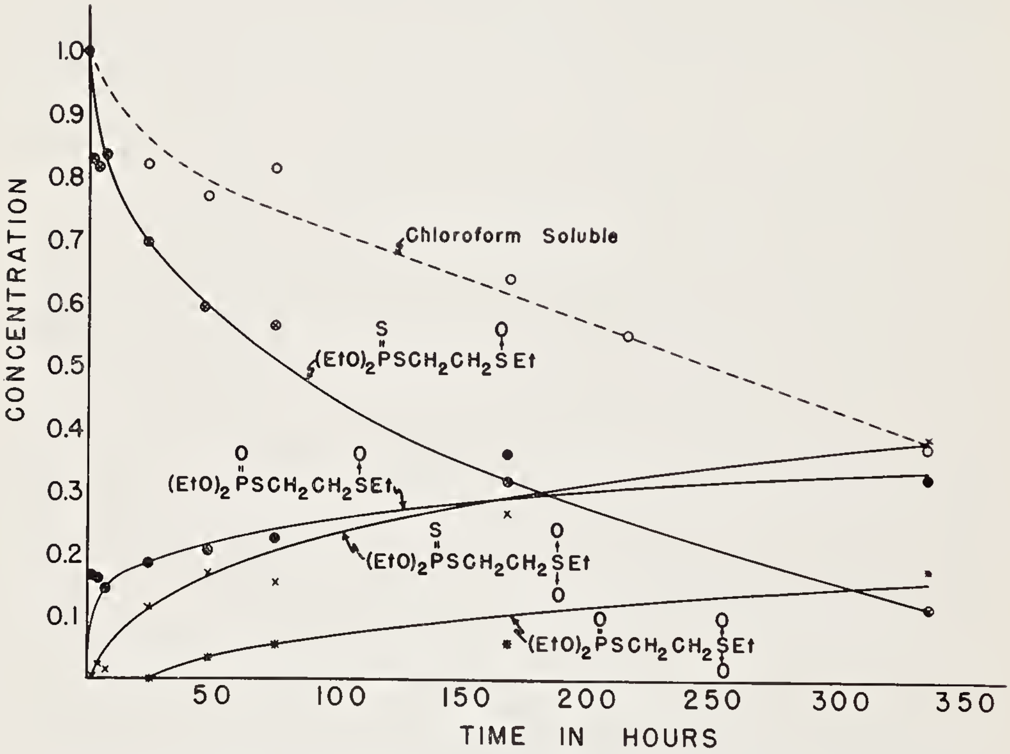


Fig. 1. Graph showing relative amounts of Di-Syston metabolites as fractions of total chloroform-partitioning P^{32} in extracts of isolated cotton leaves at various intervals after treatment. The values for the chloroform-soluble material represent the fraction of total P^{32} which partitioned with chloroform (from Metcalf, Fukuto, and March (98)).

Casida (17) that Thimet behaves in a completely analogous fashion to Di-Syston, but somewhat different rates of metabolism are exhibited (98). The relative rates of these metabolic conversions in cotton leaves are shown in Figs. 1 and 2.

Similar metabolic patterns have been reported for methyl Systox thiol isomer (103) and for Systox thiol isomer when studied alone (not in presence of thiono isomer) (44).

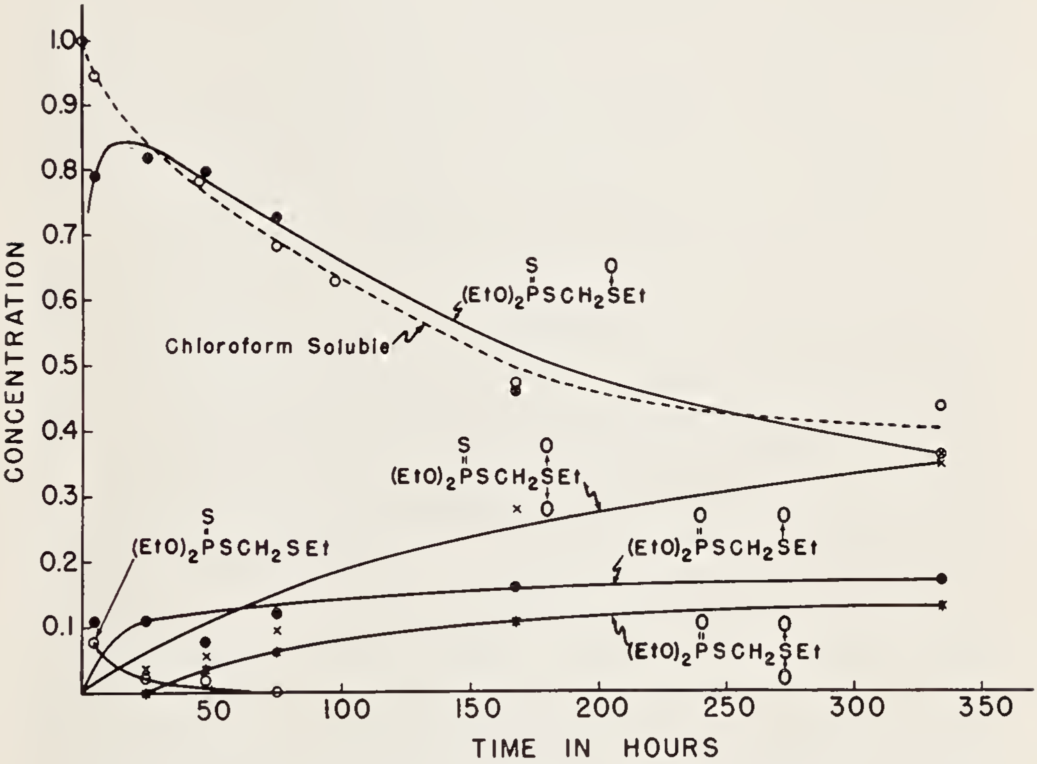


Fig. 2. Graph showing relative amounts of Thimet metabolites as fractions of total chloroform-partitioning P^{32} in extracts of isolated cotton leaves at various intervals after treatment. The values for the chloroform-soluble material represent the fraction of total P^{32} which partitioned into chloroform (from Metcalf, Fukuto, and March (98)).

Preliminary field testing of seed treatment with systemic insecticides yielded data which indicated that temperature may influence the metabolic rate of endometatoxic systemic insecticides within the plant (114). Subsequently, Metcalf *et al.* (100a) made a quantitative study of this phenomenon in several plant species with P^{32} Di-Syston. Metabolism was found to occur much more rapidly at high than at low temperatures; the increased rate was presumed to be correlated with accelerated physiological processes within the plant as a result of higher temperatures. Interestingly, different plant species exhibited different rates of metabolism; for example, the rate in cotton plants was much slower than that in tomato plants. Metabolism occurred so rapidly in the latter species that the presence of toxic metabolites was of fleeting duration, and theoretically at least, minimal toxic action could be expected in the field in this plant.

Of particular interest is that Di-Syston (98) and Thimet (17,98) are oxidized almost immediately after plant absorption so that checking at intervals of a few days to a month after application revealed the presence of the four oxidation products, but very little or none of the parent compound was found. This has also been found to be the case with other endometatoxic insecticides (55,99,103).

There are a number of insecticides which seem to lie in a position intermediate between the completely nonsystemic and true systemic insecticides. The common concept of systemic or endotherapeutic insecticides is that they are toxicants which, when applied to a plant, are absorbed and translocated in amounts sufficiently large to make the site of translocation toxic for a time to insects feeding thereon. It has been shown that many insecticides have the ability to penetrate the plant. In fact, Metcalf (96) pointed out that almost all organic insecticides are capable of penetrating into plant tissues to some extent, and effective systemic action is more a matter of degree rather than a specific property. Ripper (117) used the designation *translocated and stored in insecticidal quantities* to distinguish between systemic and merely penetrating insecticides. Perhaps this should be amplified to include *when applied in realistic amounts* because some penetrating insecticides are known to translocate but, under normal circumstances, not in amounts sufficient to make the site of translocation toxic to feeding insects. Unterstenhöfer (136) used the term "Tiefenwirkung" to designate insecticides with this type of activity. The ability to penetrate may not be particularly important insecticidally in the case of these compounds, but certainly it is an important consideration in determination of residues on a food crop. Furthermore, penetration and persistence may be specific to certain crops as illustrated by the results obtained by Anderson and Gunther (2) in the case of nicotine on certain leafy vegetables.

There are a number of insecticides for which the ability to penetrate into the plant has been established. Undoubtedly other compounds could be added to this list, but those reported in the literature are: parathion (3,24,41,59,108,130), Hercules 528 (121), Guthion® (102), Diazinon® (46), rotenone (45), quassia (3), nicotine (2,30), and dieldrin (48,63). Ripper (117) lists malathion as well. In addition, Anderson (1) found considerable penetration into the edible portion of certain root crops, particularly carrots, following soil applications with aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, lindane, and toxaphene. There was no translocation into the aerial portions of the plant, however. Terriere and Ingalsbe (133) reported similar findings and added EPN to this list. Kozlova and Dvortzova (80) found plant penetration and translocation with DDT. For the most part the above-mentioned insecticides do not warrant further consideration as systemic insecticides in this paper; however, benzene hexachloride, particularly the gamma isomer (lindane), present a somewhat different picture. That this compound has the ability to penetrate into and translocate within the plant has been established for some time, and perhaps further evidence is presented by the many reports of this material causing flavor changes in edible portions of plants (15,20,24,37,48,50,51,63,69,79,80,89,124,125, 130,133). Judging by the high rates of applications necessary for this material to translocate in toxic amounts, it must be assumed that this compound is not highly efficient as a systemic.

Of the many papers concerned with the systemic nature of benzene hexachloride, Bradbury and Whitaker (18) presented the most complete quantitative data on its absorption and translocation. These authors noted a similarity in the ability of the gamma isomer of BHC to penetrate plant and insect tissue, which had been previously demonstrated quantitatively by Armstrong *et al.* (11,12). In seed treatment a steady level of insecticide concentration is quickly attained in the outer layer while the inner concentration increases gradually (18). This is apparently due to the outer, presumably waxy layer of seeds because the rate and extent of absorption are increased markedly if the superficial, waxy layer is first removed with chloroform. Consequently, the rate of absorption varies greatly with different types of seed, presumably related to the amount and nature of the epicuticular wax and internal lipoids of the seed. Of the seed tested, linseed absorbed the greatest amount and wheat the least. They noted that the size and shape of the seed, which affect the ratio of surface to volume, are likely to influence the rate of BHC uptake. Also BHC absorption was more efficient when seedlings were grown in aqueous solutions than when the material was placed on seed. Seedlings apparently do not have the ability to convert the insecticide to water-soluble products, and loss of BHC from the plant must be ascribed either to volatilization of the unchanged insecticide or to a volatile decomposition product. They assumed that water taken up by the plant carries in the dissolved insecticide and that loss of water through transpiration is accompanied by loss of insecticide by volatilization. They conclude that the main effects of γ-BHC seed dressings are due to its systemic nature. Further evidence of BHC loss through transpiration was presented by Haines (51), who found with bioassay techniques that guttated liquid was toxic to *Aedes aegypti* larvae.

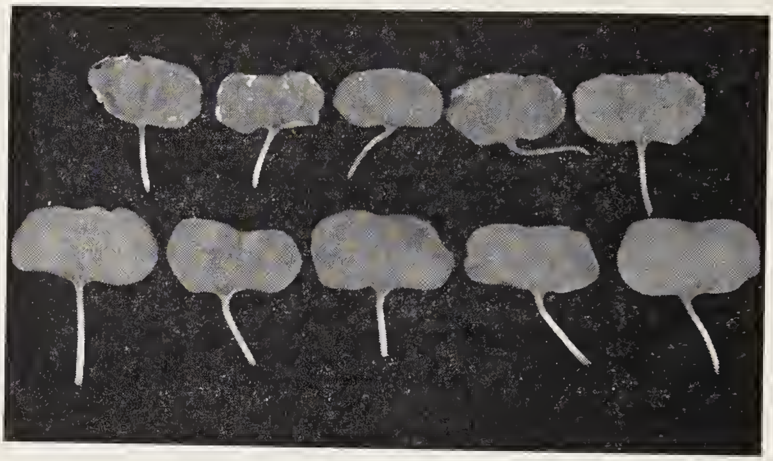


Fig. 3. Top row, cotton cotyledons illustrating typical systemic burn on outer margin and reduction in size as compared with cotyledons from untreated cotton on bottom row.

Organophosphorous systemic insecticides absorbed by roots are transported to all parts of the plant growing above ground. Movement is in the sap stream of the xylem tissue and follows the route of the plant nutrients (117). Tietz (135) and Hanna *et al.* (52) found that although there is some lateral diffusion within the plant, it is so limited that large plants, such as trees, require a circular soil application around the trunk if all aerial portions are to receive translocated material in toxic amounts (16,52).

When the plant derives the systemic insecticide from the soil, the material accumulates in the peripheral portions of the leaves, which act as blind ends of the transpiration stream (117), and consequently phytotoxicity on the leaves starts at the outer margin. This observation was substantiated by Parencia *et al.* (107) on cotyledons of cotton following seed treatment with Thimet and by Reynolds *et al.* (114) on cotton, alfalfa, and sugar beet cotyledons following seed treatment with Thimet and Di-Syston. Phytotoxicity with this latter compound was less pronounced than with Thimet in all cases.

The mechanism of absorption of the systemic insecticides by the roots seems to be variable. Ripper (117) suggests that two processes are involved. His suggestion appears to be based largely upon results reported by David (28,29) and Tietz (135). David found that roots of the broad bean plants selectively absorbed dimefox (Hanane®) from solutions (29) at rates of transpiration at which schradan was selectively rejected (28). Systox is absorbed by the root over a period of time until the concentration inside the root is higher than that outside. Ripper (117) concludes, therefore, that (*a*) absorption may take place by a passive movement into the root and thus into the transpiration stream as may be the case with schradan and (*b*), in the case of dimefox or Systox, absorption into the root proceeds until the concentration is higher inside than outside the root. The latter process takes place in detached roots, and Tietz (135) suggests that this is due to the lipoid solubility of Systox. He found that roots of *Phaseolus* plants placed in Systox emulsions, show greater root permeability than those placed in water and suggests that this may be due to increased respiration.

In order to study toxicant distribution within plants grown from seed coated with systemic insecticides, radiotracer techniques were utilized. Seeds of cotton, sugar beet, and alfalfa were coated with synthesized P^{32} preparations of Thimet, Di-Syston, and thiol isomer of Systox. In all three plant species the highest level of radioactivity was found in the cotyledons, and it was not subsequently translocated in substantial amounts to the true leaves. To become toxic to insects, therefore, newly formed leaves must depend upon continuous absorption and translocation from the roots. Thus, it was found that the toxicant within the plant ranges in a concentration gradient with the highest value in the oldest leaves and the lowest in the youngest (114).

It has been demonstrated that root absorption of systemic insecticides from sand and to an even greater extent from rich, colloidal soils is not as efficient as when roots are placed in aqueous systemic solutions. Based on evidence gathered on trees, Hanna *et al.* (52,53) suggest that soil applications are not as efficient as bark treatment (16,73) because adsorption to soil particles, leaching, evaporation, and decomposition by soil organisms are all factors in reduced efficiency in soil application. Reynolds *et al.* (114) suggested leaching as a factor in loss of seed treatment efficiency when excessive irrigation water was used. This conclusion was based upon the fact that when excessive irrigation water was used in several fields to germinate sugar beet seed coated with systemic insecticides, there was no phytotoxic effect on the cotyledons and no mortality of beet army worm, *Laphygma exigua* (Hbn.), larvae. In other fields, which did not receive excessive water, cotyledon injury was apparent, and there was approximately a 50% reduction in populations of beet army worm larvae.

B. SEED TREATMENT

Seed treatment with systemic insecticides has in the past consisted largely of soaking the seeds in organophosphorous systemic solutions or of coating the seed with lindane. Recently, interest and research have been directed toward other methods of treating seed, particularly that utilizing impregnated powder coatings.

The use of lindane, or the gamma isomer of benzene hexachloride, for seed treatment is discussed in Sections II.B and III.A. It is difficult to designate with any certainty the particular method by which this compound affects plant protection. Very likely the three methods of activity—contact, repellent, and systemic—are closely interwoven in plant protection. Bradbury and Whitaker (18) stated that the primary toxic action seemed to be of a systemic nature. This would certainly be the case in control of adult flea beetles, *Phyllotreta sp.*, as it is practiced in several areas of the world, particularly Great Britain (5,6,69). Dosages are considerably higher for flea beetle control (0.25–1.5 ounces of 75% gamma isomer per pound of seed) than those commonly used in California for seed-corn maggot and wireworm control (approximately the same amount of material per 100 pounds of seed; see Table I) which may explain the lack of noticeable systemic activity in the latter case. Dosages as high as these give protection from flea beetle attack for a period of several weeks. However, many varieties of seed will not tolerate high dosages, and a recent communication from Great Britain indicates occasional germination difficulties in that country with grain, though seldom with cruciferous seed.

Several early papers reported that seeds soaked in organophosphorous systemic insecticides were penetrated to the extent that the ensuing seedling plant was toxic to certain insects (23,66,68,113,115,118). David and Gardiner (32) presented a detailed report on the factors which influence the efficiency of seed soaking with Systox. They found that with large seeds, such as broad bean, the ensuing plants are quite toxic to aphids, but with small seeds the effectiveness is considerably reduced; hence, effectiveness is proportional to the amounts absorbed. Because there was considerable variation in the rate of absorption among individual seeds of the broad bean, a 24-hour period of soaking was necessary. With Systox solutions equal amounts of water and Systox were absorbed. After short periods of soaking (4 hours) there was more insecticide in the seed coat than in the cotyledons, but after 24 hours of soaking there was more material in the cotyledons. Even with the soaking technique some material was lost into the soil after planting, but a certain proportion of it was subsequently picked up by the roots. When the same amount of toxicant was used, more was absorbed by the plant from seed soaking than a similar amount applied by soil drenching. Seeds soaked, dried, and stored for one month produced plants toxic to aphids.

These authors (32) reported that cabbage seed soaked for 4 hours in a 1.0% solution of Systox, or for 24 hours in a 0.2% solution, was not toxic to *Myzus persicae* 25 days after soaking, when infestation was first attempted. Somewhat similar results were obtained with other small seeds tested. Good results were reported when large bean seed or potato tubers were soaked. Reynolds *et al.* (113), after soaking cabbage seed for 24 hours in a 1.0% solution of schradan and a 0.5% solution of Systox, reported similar results in that some control was apparent but of a short duration in both cases. The problem, then, with small seeds is getting enough in the seed for effective results.

On the other hand, in the case of alfalfa seed soaks, Reynolds *et al.*

(114) reported good results in control of the spotted alfalfa aphid, *Therioaphis maculata,* when the seed was soaked for 3 hours in a 0.25% solution of Systox. This aphid has been shown to be quite susceptible to insecticides; Thimet, Di-Syston, and thiol isomer of Systox were toxic to it in concentrations of less than 10 p.p.m. within the plant (114). However, alfalfa seed soaked similarly in a 0.25% solution of Tetram® (R-6199, or oxalate salt of *O,O*-diethyl *S*-2-diethylaminoethyl phosphorothiolate) gave poor results, which, it is reasoned, were due primarily to the fact that this material is not translocated in substantial amounts from the point of application (101). Tetram has since been shown to be a relatively inefficient aphicide (132).

There are several advantages and disadvantages in the use of seed soaks with systemic insecticides. In brief, the advantages are that it is economical of material (32,114) and is a simple method of treatment for small quantities of seed (32). The disadvantages seem more important, however. The amount the seed will tolerate without impairment of germination is limited (32). Wet seeds are difficult to sow and so must be dried; this creates a mechanical problem and increases costs (32,114). In general, soaking is most effective only with large seeds (32). Some seeds germinate almost immediately after soaking (114).

An alternative to soaking is coating the seeds. David and Gardiner (32) and Ivy (66) pointed out the advantage of coating the seed with an impregnated powder over soaking; that a seed can carry much more material on its surface than the embryo can tolerate under full absorption through soaking. Activated charcoal was used successfully (14,66), and Ivy (66) stressed the fact that larger amounts of toxicant in this carrier could be used with little phytotoxicity. Kantack (74) tested several materials as systemic carriers for seed coating and selected carbowax 6000, a water-soluble polyethylene glycol with a melting point of 60–63°C., as the most promising. In germination tests, 0.5 pound of the toxicant blended with 4 pounds of carbowax 6000 was added to a bushel of wheat seed without reducing germination. Systemic materials tested were Systox, methyl Systox, schradan, *O,O*-diethyl *S*-isopropylmercaptomethyl phosphorodithioate (compound 12008), and *O,O*-diethyl *S*-*n*-propylmercaptomethyl phosphorodithioate (compound 12009). Reynolds *et al.* (114) tested Attaclay as a carrier but found it unsatisfactory because it could not carry 50% by weight of the liquid technical materials under test (this percentage was considered the minimum for satisfactory adherence of a powder to smooth seed coats at the high rates necessary for good field results). Consequently, activated charcoal was selected as the carrier for further testing.

Methyl cellulose aids materially in adhering the charcoal formulation to the seed (114). It may be added at the rate of 2–4% by weight of charcoal formulation or, preferably, dissolved in water to make a 2–3% solution (114). Of several methods tested, such as slurry and liquid-fixation, a modified liquid-fixation where the seed is dampened and the activated charcoal formulation added was settled upon as the most convenient (114). On some seeds with a fuzzy seed coat, such as encountered in mechanically delinted cotton seed, or a rough seed coat, such as sugar beet seed, it is not necessary to add moisture to stick the charcoal formulation to the seed if they are tumbled together for some time. On seeds with smooth seed coats, such as alfalfa or acid-delinted cotton seed, water and a suitable seed adhesive must be added (114).

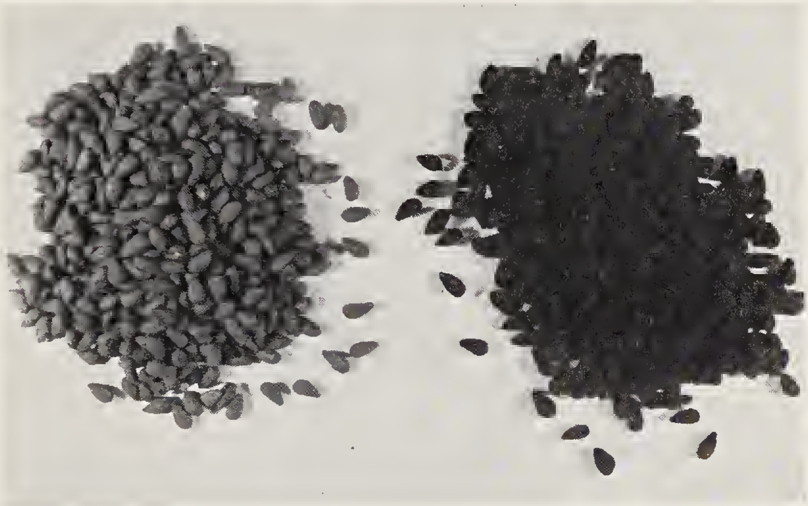


Fig. 4. On left, untreated acid-delinted cotton seed; on right, acid-delinted cotton seed treated at rate of 2 pounds of 50% Thimet on activated charcoal per 25 pounds of seed.

Emulsifiable formulations were tested in which only enough water was added to distribute the material uniformly over the seed (114). This is little more than a modified seed soak which was discussed previously. On alfalfa, rates up to 0.5 pound of toxicant per 25 pounds of seed had no adverse effect on germination. The small seeds were left too sticky to flow well in the seeding process, and it was theorized that an absorptive powder could be added after treatment to mitigate the sticky characteristic. On cotton, however, emulsifiable formulations caused a reduction in germination, and the method was abandoned (114). On sugar beet seed impregnated powder coatings were less damaging than emulsion treatments (57).

In P^{32} radiotracer studies in the greenhouse, seed of cotton, alfalfa, and sugar beets were treated with identical amounts of Di-Syston, Thimet, and thiol isomer of Systox by three methods of treatment: activated charcoal seed coating, emulsion seed coating (see preceding paragraph), and placing granules in the seed furrow. Data obtained from this experiment indicated surprisingly small differences in rates of absorption and translocation of the toxicant among the three methods of treatment. Although not conclusive from data obtained, it appeared that the emulsion coating was a little more rapidly absorbed and the granule application persisted for the longest period. This trend was borne out in subsequent field experiments and in residue studies on alfalfa (114).

Activated charcoal formulations have presented certain difficulties both in the coating process and during the subsequent planting. The rate of planting of the coated seed is less than that of untreated seed (8,10,107,114). Reynolds (112) noted reductions up to 30% in planting rates in the case of cotton that had been treated at the rate of 2 pounds of the 50% activated charcoal per 25 pounds of seed and up to 12% reductions with half this rate. Growers must calibrate their planting equipment and compensate for the reduction in planting rate in order to plant the desired amount of seed per acre. This problem is undoubtedly more pronounced in the case of large seed because, when treated at the same rate of toxicant per seed weight, the individual seed of large varieties carries a much thicker coat. This was very apparent when cotton and smaller alfalfa seed, treated at identical rates, were compared.

There are mechanical problems in coating small seed such as alfalfa seed. It is difficult to obtain a firm coating and uniform distribution of the activated charcoal on the seed, which will not come off in subsequent handling after treatment, at the high rates necessary. Most seed-treating devices available commercially at the present time are not satisfactory for applying the heavy charcoal coatings necessary. This seems to be the case particularly with equipment used commercially in making slurry applications. The water may be applied to the seed either before or after the addition of the impregnated activated charcoal. It is then necessary to tumble or mix the seed for some time with the material. In general, with small seeds, it is preferable to treat a fairly large amount at one time, as the combination of seed weight and tumbling action not only distributes the material but tends to pack or compress the charcoal onto the seed. Packing the material onto the seed, combined with the action of the methyl cellulose adhesive, should give a durable coat which will not subsequently break off in substantial amounts. Also when large amounts are treated, the weight of the seed tends to inhibit "clumping," or formation of balls, of this small seed and reduces the tendency of small seed, when wet, to stick to the various components of the treating device. It is usually necessary to sack the seed when still slightly damp, since continued mixing when dry tends to break off the coating.

On large seed such as cotton, coating techniques are simplified. The size and weight of the individual seed are such that there is little difficulty in obtaining a durable seed coat. Simple devices, such as cement mixers or insecticide bait tumblers, are entirely satisfactory. There seems to be little doubt, however, that, in view of the extreme toxicity of the organophosphorous systemic insecticides currently under investigation, commercial seed companies with suitable equipment must assume the responsibility for seed dressings.

There is a need for improved techniques in coating seed, but suitable equipment can be designed for available toxicant carriers such as the activated charcoal. As previously stated, methyl cellulose aids in adhering charcoal formulations to the seed. Certain polyvinyl compounds, when tested as adhering agents, gave seed coats of better durability than those obtained with methyl cellulose. The most promising types of seed coats currently under investigation, however, are ones which can be applied dry but which do not rub or break off the seed in substantial amounts during handling prior to and during planting. Insoluble powder coatings may not be ideal, however, perhaps a more desirable carrier would be a water-soluble powder, containing 50% or more toxicant, which, after drying on the seed, would leave a hard, smooth surface. When the treated seeds are planted, this coating would dissolve or readily permit soil moisture penetration to the seed in order that good germination will result.

There have been some difficulties in obtaining good seed germination in field plantings of cotton, the reasons for which are not completely understood at this time. Parencia *et al.* (107) reported a pronounced reduction in plant emergence when the field received a heavy rain the day following planting. Seed in this field had been coated with 50% Thimet on activated charcoal and planted at a rate to give 1 pound of toxicant per acre. It is presumed the seed was treated at approximately the rate of 2 pounds of the 50% material per 25 pounds of seed. There has been more trouble on heavy clay soils, insofar as germination is concerned, than on lighter, sandy soils (8). In laboratory studies it was found that planting when soil temperatures are low, particularly when soil moisture is high, may aggravate germination problems (107). In recent greenhouse studies with nonsterile field soil at low temperatures (61–64°F.), a Thimet 44D seed treatment (2 pounds per 25 pounds of seed) on cotton seeds previously treated with Ceresan 200 considerably reduced plant emergence. At soil temperatures above 73°F., this effect was not as evident. A part of the reduction of emergence was due to a partial inactivation of Ceresan 200 by the charcoal carrier in Thimet 44D, but a greater part appeared to be due to a depressing physiologic effect of the compound on the seedling plant vigor which rendered the seedling more susceptible to disease. The depressing effect of Thimet was not more than mildly evident on pathogen-free soil but occurred consistently in field soil or soil infested with pathogenic *Pythium spp.* Captan, when substituted for Ceresan 200 in combination with Thimet, has induced satisfactory emergence of cotton in field soil in greenhouse studies, but in field studies results have not been consistent (39a,39b). It was shown in the case of sugar beets that, when soil moisture is barely adequate to germinate untreated seed, germination of coated seed was reduced, but a subsequent irrigation provided adequate moisture and many treated seeds germinated at this later date (114). It was suggested that perhaps the activated charcoal may compete with the seed for available moisture, and under conditions of low soil moisture germination may be reduced (114). Delays of 24–36 hours in germination of treated seed have been reported (8), and it is possible the delayed germination may be due, at least partially, to the heavy charcoal coating retarding moisture penetration to the seed rather than being due entirely to the toxicant. Under good germination conditions in the field, plants grown from seed coated at the approximate rate of 1 pound of toxicant per 25 pounds of seed showed no appreciable loss of stand in the case of cotton with Thimet (107,114) and Di-Syston (114), alfalfa with Thimet and Di-Syston (114), and sugar beets with Thimet (57,114) and Di-Syston (114). Systox and thiol isomer of Systox on all three crops at 4 ounces of toxicant per 25 pounds of seed did not impair germination (114). Under greenhouse conditions Hills *et al.* (57) recorded some difficulty with seed viability when sugar beet seed was coated with Thimet and compound 12008 at the rate of 0.75–1.0 pound of toxicant per 15 pounds of seed. The effects were not noticeable in field plantings, however. In another area dosages as high as 2 pounds of Thimet and Di-Syston per 25 pounds of seed did not impair germination of this crop in the field (114). Germination of soybean seed (Lee variety) and sorghum seed (Ryer 15 variety) was badly impaired with Thimet and Di-Syston seed dressings, although it was less serious with sorghum than with soybean and Di-Syston was not as damaging as Thimet (114). From the foregoing discussion it would appear that different seeds vary considerably in their tolerance to seed treatment with organophosphorous systemic insecticides. Furthermore, such a reasonably tolerant seed as cotton, when subjected to adverse germination conditions, may yield a reduced plant stand.

Seed coatings with systemic insecticides are effective on a surprisingly wide range of insect and spider mite pests, particularly with the two newly developed materials, Thimet and Di-Syston. The majority of information developed to date has been on cotton and to a lesser extent on alfalfa and sugar beets. Information on other crops, particularly vegetables, is rather meager at the present time.

Coating alfalfa seed with systemic materials prior to planting resulted in good control of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), for several weeks after planting. Systox, thiol isomer of Systox, Di-Syston, and Thimet all gave good results and were superior to thiol isomer of methyl Systox. There were indications that Systox, particularly the thiol isomer, was absorbed and translocated somewhat more quickly than Thimet and Di-Syston (114). This agrees with previous work which showed that the thiol isomer of Systox is initially absorbed and translocated 1.5–2 times as fast as Thimet and Di-Syston (98). It was suggested that the explanation lies in the comparative water solubilities of these compounds, since thiol isomer of Systox and, to a lesser extent, the thiono isomer form oxidative metabolites which are more water-soluble in the subcuticular layers of plant tissue. For effective control in the field it appeared necessary to use from 8 to 16 ounces of toxicant per 25 pounds of seed; consequently for reasons of practicality, further work was concentrated on Thimet and Di-Syston (114). Of these two compounds, Di-Syston was slightly superior for spotted alfalfa aphid control; when the rates were similar, Di-Syston was effective for about one week longer than Thimet. When seed was treated at the rate of 1 pound of toxicant per 25 pounds of seed, the effective period of control was about 5–6 weeks when planting was done at a time of year when alfalfa grew rapidly, and 9–10 weeks when planting occurred at a time of year when the plants grew slowly. In either case effective aphid control ceased when the plants attained a height of about 6 inches. Therefore, it was suggested that the rapidity of metabolism to non-toxic products within the plant may be at least partially dependent upon the rate of plant growth. Coating the alfalfa seed at 0.5 pound of toxicant per 25 pounds of seed reduced the effectiveness somewhat, but not to a large extent (114).

Work conducted in desert areas indicates that coating sugar beet seed with systemic insecticides is effective in killing the curly top virus disease vector, beet leaf hopper, *Circulifer tenellus* (Baker), for a period of 2–3 weeks after emergence of the seedling plants (40,57). By this time the plants have reached approximately the 4–6 leaf stage, and at this stage of growth the varieties of sugar beets grown in these areas attain a degree of tolerance to the destructive curly top virus. This treatment will not protect the plants from attack by leaf hoppers which migrate into the field, but may reduce curly top incidence by killing the leaf hopper migrants before they can feed on and thus infect a large number of plants. This desirable protection has not been proven as yet, but preliminary data obtained under conditions of very low leaf hopper populations indicate some reduction of virus-infected plants (40). In view of the low seeding rate of sugar beet seed, 5–8 pounds of seed per acre, seed treatment at the rate of 1 pound of toxicant per 25 pounds of seed should not be costly to the grower. Results from planting sugar beet seed which had been coated with Thimet and Di-Syston at the rate of 1 pound and, with Systox at 0.25

pound, per 25 pounds of seed showed Thimet to be the most effective material and Systox to be the least effective at these rates (114). Hills *et al.* (57) found Thimet and compound 12008 to be effective for 2 weeks after plant emergence. Some protection of beet plants from attack by lepidopterous larvae (primarily beet armyworm, *Laphygma exigua* (Hbn.)) was reported, but the reduction was not considered particularly promising (57,114).

Information concerning effectiveness of systemic seed treatment on cotton is available on a wide variety of seedling pests, although much of it is of a preliminary nature. Although toxic to about the same range of pests, experiments with compounds 12008 and 12009 showed these materials to be somewhat less effective than Thimet (107). Other workers having reported similar results, interest has largely shifted from these compounds to Thimet and Di-Syston. For reasons of economy, it is not practicable to apply Systox or its thiol isomer at rates comparable to those of Thimet and Di-Syston; thus, although the former compounds are known to be effective as seed treatment on certain pests (114), their effectiveness at rates as high as those used for Thimet and Di-Syston is not fully known. Parencia *et al.* (107) reported seedling cotton plants grown from seed treated at the approximate rate of 1.0 pound of toxicant per 25 pounds of seed to be toxic to thrips for about 5 weeks and to aphids for several weeks; in addition, leaf miners did not develop in cotyledons of treated plants. Thimet at this rate was not effective for the desired period of time against cotton flea hoppers and boll weevils, although some initial control was observed. Reynolds *et al.* (114) reported Thimet at 1.0 and 0.5 pound per 25 pounds of cotton seed to be effective on thrips for about 4–5 weeks with little difference between the two treatments. At the higher rate, plants from Thimet-treated seed were toxic to aphids for about 5–6 weeks and at the 0.5 pound rate for almost as long. Thimet seed treatments were reported as promising on control of leaf miners, cutworms, flea beetles, white flies, and leaf hoppers (114).

Thimet seed treatment at 1 pound per 25 pounds of seed was reported to control spider mites from 4–6 weeks (9) to as long as 9 weeks (8). Reducing this dosage by half, the effective period of control was reduced in the case of thrips, aphids, and spider mites by about 1–2 weeks (9). Reports listed darkling ground beetles (*Blapstinus sp.*) and cotton leaf perforator (*Bucculatrix thurberiella* Busk.) as being susceptible to Thimet seed treatment (8,9,114).

In general, results with Di-Syston, were strikingly similar to those obtained with Thimet on the same pests (9,10). Reynolds *et al.* (114) noted that this material was slightly more effective than Thimet for control of cotton aphid but less effective for control of flea beetles.

Kantack (74) reported satisfactory reductions of wheat curl mite, *Aceria tulipae* (K.), on young wheat plants with seed coatings at 0.5 pound per bushel of seed with Systox, schradan, methyl Systox, and compounds 12008 and 12009.

On vegetables, particularly those with tubers or large seeds, Thimet coatings have shown promise against aphids, spider mites, leaf hoppers, thrips, seed-corn maggot, and a few other pests (8); however, few detailed reports have appeared to date.

C. GRANULAR APPLICATION AT TIME OF PLANTING

In comparison to seed treatment, few reports have appeared concerning granular applications of systemic insecticides applied at time of planting.

In control of spotted alfalfa aphid on seedling plants granular application of Di-Syston and Thimet have given excellent results (114). It was shown that systemics applied as granular formulations may be absorbed and translocated a little more slowly than seed treatments, but the residual period of activity was somewhat longer, perhaps one week. Alfalfa seed was broadcast in the tests reported, and it was shown that the granules may be broadcast at the time of seeding or afterward as long as the granules are harrowed or cultipacked in with the seed prior to irrigation. If the seed is drilled into the soil, the granules may be drilled in with the seed through a fertilizer attachment (114).

When 2% granules of Thimet or Di-Syston were applied at the rate of 50 pounds per acre (1 pound toxicant) in the seed furrow while planting sugar beet seed, the resulting control of beet leaf hopper was quite similar to that obtained with seed coatings of the same materials when applied at the rate of 1 pound of toxicant per 25 pounds of seed. When the seeding rate of 5–8 pounds of seed per acre is considered, however, it is obvious that the seed coating is much the more economical of the two methods (114). Application of Thimet granules in greenhouse studies resulted in some stunting of sugar beet plants (47), but there was no injury in field trials (114).

Applications of granular formulations of Thimet have shown promise when applied to cotton at time of planting for control of seedling insects (8).

The potential of granular applications of systemic insecticides applied at time of planting appears to be great. On very small seed it is doubtful that the heavy seed coatings required with the materials currently under investigation and with the current methods of coating can be applied satisfactorily. As granular formulations are efficient in absorption and translocation, they may be found to be more practical than seed coatings in the case of very small seed. In addition, certain seed are known to be relatively intolerant of seed coating with Thimet and to a lesser extent with Di-Syston, and it has been demonstrated in the case of intolerant seed that granules do not cause as severe impairment of germination as do seed coatings (114). In order to use systemic applications at planting time on certain seed, therefore, granules may be a safer and more practical method of application.

Granular applications made at time of seeding in cold frames or hothouses should give good protection against certain insects, particularly aphids, even for a short time after transplanting into the field. Although not strictly an application at planting time, certainly granular applications made at time of transplanting certain crops would appear promising. This would not hold true on many crops, but in the case of cruciferous crops, for example, this should alleviate problems of cabbage aphid control for some time after transplanting. The granular formulation could be applied in the transplanting hole prior to placing the plant or side-dressed with a fertilizer attachment. In fact, insecticide-fertilizer mixes for control of soil insects are utilized commercially in many areas at this time, and there seems to be no reason why applications at planting time of fertilizer--systemic insecticide mixes may not be just as promising for investigation in control of other pests. The same general procedures for placing fertilizers properly so as to make them available to the roots would seem to apply in the case of systemic insecticide granules; however, the granular insecticide, being insoluble, may need to be placed closer to the roots.

D. LIQUID APPLICATION AT TIME OF PLANTING

Liquid applications applied at time of planting have not been investigated as fully as desirable. That they are effective has been amply demonstrated. It was shown on alfalfa that broadcast sprays of Thimet and Di-Syston, applied after seeding and prior to cultipacking or harrowing in the seed, were very effective in controlling spotted alfalfa aphid for several weeks after plant emergence (114). As in other cases previously mentioned, Di-Syston was somewhat superior in these tests to Thimet for aphid control. Probably the most practical method of applying liquids to row crops at planting time is by a soil spray or stream. This may be accomplished by arranging a spray nozzle after the planting shoe so that the liquid is applied in the seed furrow. Ripper (117) pointed out a recent refinement of this method in which the seed is placed by a conventional seed drill, the seed furrow is partially filled with soil, the systemic application is made in a 2-inch band, and finally the seed furrow is filled with soil by dragging it in with a chain just before consolidation with a compress wheel. Such an application would mitigate impaired germination, but effectiveness would have to depend on crown absorption or leaching to the smaller rootlets. Manual applications of systemic solutions do not appear practical even under planting procedures in which the seeds are planted in hills or where points of seeding in the row are not continuous.

IV. Application of Systemic Insecticides to the Soil after Planting

Application to the soil after planting time is not actually within the scope of this review. Ripper (117) in his commendable review covered most of the available literature in a very thorough fashion. As discussed previously, it has been shown in the case of trees that soil applications are less efficient than bark treatment (16,52,53,73). In all likelihood a similar situation exists with other large plants. In the case of large cotton plants relatively large doses of systemic materials were required for control of spider mites, which are highly susceptible to organophosphorous systemic insecticides, and the resulting control was slow in taking effect (126). At best it would appear on large plants that for reasons of economy with the presently available systemic insecticides, applications will be limited to the more susceptible pest species. The greatest potential, then, would appear to be on smaller plants, particularly vegetables and ornamental plants. Ripper (117) points out that soil applications of dimefox are used in England for control of aphids on sugar beets and mangolds. Dimefox applied to the soil has given good aphid control on hops (117), as has Thimet (31). Similar results have been reported on crucifers (113) with soil treatment.

The potential would appear great in the use of soil applications on greenhouse and nursery plants. Hazards in applications would be minimized; and, as greenhouse plants are notoriously susceptible to foliage injury, application through the roots may be feasible. Many pests on ornamental plants are difficult to contact with conventional insecticides, but no phytophagous pest can escape exposure through soil application. The newer systemic insecticides, through their wide range of effectiveness, may prove immensely valuable.

V. Residue Problems

Persistent residues resulting from prolonged absorption by the roots limit the use of systemic insecticides applied to the soil at the present time. On nonfood crops, such as ornamentals or crops grown for seed production only, there is obviously no problem. On food crops there is no reason to believe that residue problems are greater with systemic insecticides than with conventional insecticides, once the residue problem is understood and proper analytical procedures are initiated. It seems highly desirable, however, to establish degradation or persistence curves wherever possible. It may be necessary to establish persistence curves on some crops under different growing conditions, as it has been suggested that the rate of metabolism within the plant may vary when subjected to different growing conditions (114). Area differences may be important, for it is conceivable that variable cultural practices in crop production may affect persistence of root uptake and metabolism within the plant. Perhaps residue persistence from irrigated and nonirrigated areas will be found to differ. Many other factors could affect persistence in the soil as well as within the plant. For example, in sandy soils much more compound will be leached down and away from the root zone than would be the case in heavy soils, but as the roots grow, they may be able to tap soil areas containing additional compound, and this could create a renewed residue problem.

Fortunately, adequate methods of analysis are available at the present time. There are two methods currently in use. The first is utilization of radiotracer techniques in which P^{32} preparations are made of the compound under study, and the second is the cholinesterase inhibition techniques such as the electrometric cholinesterase procedure described by Hensel *et al.* (56).

At the present time little is known of the residue problems resulting from soil applications on most crops. Considerable information is available, however, concerning residues of Thimet on cotton and a few vegetable crops (17) and of Thimet and Di-Syston on alfalfa (114). These papers contain residue information which may be quite representative of that to be obtained on other crops in the future and are therefore reviewed here in some detail.

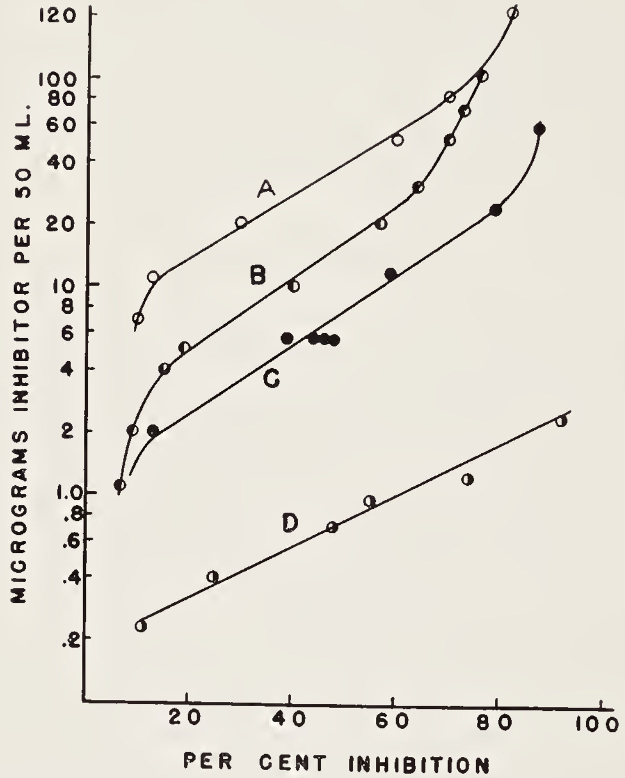


Fig. 5. Standard curves showing relative inhibition of human blood plasma cholinesterase by Thimet and Di-Syston metabolites as determined by electrometric technique. *A*, Systox thiol isomer sulfoxide; *B*, Systox thiol isomer sulfone; *C*, Thimet oxygen analogue sulfoxide; and *D*, Thimet oxygen analogue sulfone (from Reynolds, Fukuto, Metcalf, and March (114)).

Before a well-conceived residue program can be undertaken, it is essential that the characteristics and behavior of any metabolic products of the parent compound within the plant be known. As previously mentioned, in the case of *stable* or *endolytic* systemic insecticides there are essentially none within the plant; only the parent compound is present. With *endometatoxic* compounds, on the other hand, both active and nontoxic metabolites are present in the plant. Neither Thimet nor Di-Syston *per se* is a strong cholinesterase inhibitor, so detection is difficult in micro amounts. As previously discussed, however, it has been shown that both compounds are rapidly converted into potent anticholinesterase agents and after a short time relatively little or none of the parent compound is found within the plant. Consequently, standard curves showing relative inhibition of cholinesterase of the active metabolites must be determined for the crop under investigation.

As mentioned earlier, the eventual major metabolites from Thimet are the oxygen analogue sulfoxide and sulfone, *O,O*-diethyl *S*-ethylsulfinylmethyl phosphorothiolate and *O,O*-diethyl *S*-ethylsulfonylmethyl phosphorothiolate, respectively. Analogously, the eventual major metabolites from Di-Syston were found to be Systox thiol isomer sulfoxide and sulfone, *O,O*-diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate and *O,O*-diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate, respectively. Figure 5 shows the standard curves obtained with all four inhibitors (114). Micrograms inhibitor per 50 ml. are plotted against per cent inhibition. As these curves were determined for alfalfa residue studies, the curves were obtained by adding calculated amounts of inhibitor to the alfalfa water homogenates and the procedure of Hensel *et al.* (56) was followed. Accordingly, residues of Thimet and Di-Syston found in alfalfa treated at planting time by coating seed, broadcasting granules, and broadcast sprays at various intervals after plantings were calculated as p.p.m. of the respective sulfoxide and sulfone phosphorothiolates. The data obtained are shown in Table II.

It should be noted in Table II that the alfalfa was growing slowly during the period of this test. When growth was rapid, residue dissipation occurred about 2–3 weeks earlier (114). The granular application as shown in Table II was applied broadcast at a rate to give approximately 1 pound per acre. In all probability a similar amount applied to a row crop by side dressing would persist somewhat longer.

In radiotracer techniques a somewhat different problem exists.

TABLE II

Residues of Di-Syston and Thimet on Seedling Alfalfa Following Treatment at Time of Planting (114)[a]

Material	Application method	Amount toxicant	P.p.m. Systox thiol isomer sulfoxide at given time interval (days)				P.p.m. Systox thiol isomer sulfone at given time interval (days) after planting			
			32	48	62	76	32	48	62	76
Di-Syston, 50% on charcoal	Seed coat	1.0 lb./25 lb. seed	4.5	1.1	<0.1	<0.1	1.9	0.39	<0.03	<0.03
Di-Syston, 2% granule	Broadcast	1.0 lb./acre	4.7	1.1	<0.1	<0.1	2.1	0.39	<0.03	<0.03
Di-Syston, emulsion spray	Broadcast	2.0 lb./acre	2.3	1.2	<0.1	<0.1	0.90	0.43	<0.03	<0.03
			P.p.m. Thimet oxygen analogue sulfoxide at given time interval (days) after planting				P.p.m. Thimet oxygen analogue sulfone at given time interval (days) after planting			
			32	48	62	76	32	48	62	76
Thimet, 44% on charcoal	Seed coat	0.88 lb./25 lb. seed	3.0	0.21	<0.02	<0.02	0.36	0.03	<0.003	<0.003
Thimet, emulsion spray	Broadcast	2.0 lb./acre	3.06	0.48	0.03	<0.02	0.43	0.05	<0.003	<0.003

[a] Treated and planted October 26, 1956, at time of year when alfalfa grows slowly.

TABLE III

Residues of P^{32} Thimet and Metabolites in Cotton Following Seed Treatment (17)

Lb. Thimet/ 100 lb. seed	Location	Time interval (weeks)	Plant part	P.p.m. Thimet equivalent (P^{32}) present				P.p.m. phosphorothiolate sulfone equivalent[a]
				Hexane fraction	Acetone-water fraction	Hyd. product	Unextractable residue	
16	Wisc.	3.9	Foliage	0.27	0.29	0.54	0.93	0.42
16	Wisc.	7.4	Foliage	<0.0006[b]	0.0036	0.019	0.035	—
16	Wisc.	10.7	Leaves	<0.0006	<0.0006	<0.0006	<0.0006	0.0084
16	Wisc.	10.7	Squares	<0.006	<0.006	<0.006	<0.006	0.063
16	Okla.[c]	16	Cottonseed[d]		<0.025[a]	—	—	—
32	Okla.	16	Cottonseed[d]	<0.030	<0.030	—	—	<0.025
32	Okla.	16	Leaves	<0.005	<0.005	—	—	—

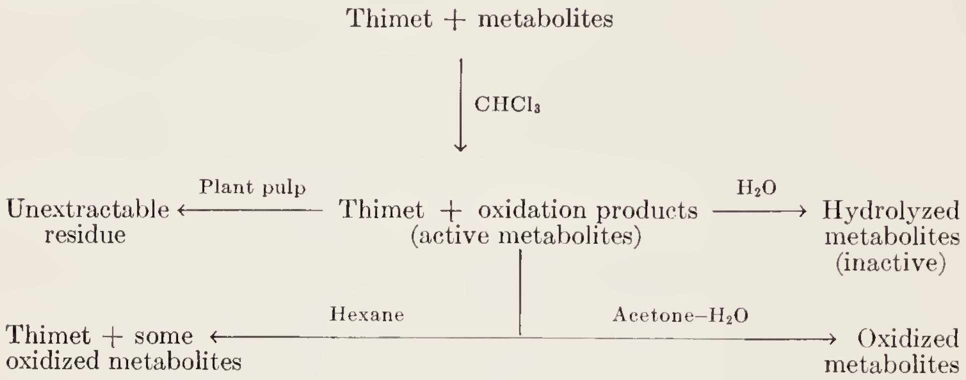
[a] Results based on anticholinesterase activity of chloroform-soluble materials, except in cottonseed oil which was further fractionated to recover acetone-water fraction for analysis. Addition of phosphorothiolate sulfone to untreated seed at 0.025 p.p.m. yielded 15% cholinesterase inhibition in acetone-water fraction and at 0.05 p.p.m. yielded 33% inhibition.

[b] Sensitivity limit of method: no radioactivity or anticholinesterase activity detectable.

[c] Cotton grown in Oklahoma through generous cooperation and assistance of R. M. Chatters and H. E. Howell, Oklahoma A. & M. College.

[d] Mature cottonseed. Figures for p.p.m. and anticholinesterase activity based on materials extractable by both ether and chloroform in a Soxhlet and expressed on basis of whole seed weight.

As the P^{32} is found in the various metabolic products, toxic and nontoxic, techniques have been developed to fractionate the various products. A convenient diagram of the procedure in the case of Thimet was presented by Bowman and Casida (17) as follows:


Thimet + metabolites
$CHCl_3$
Plant pulp
Unextractable residue
Thimet + oxidation products (active metabolites)
H_2O
Hydrolyzed metabolites (inactive)
Hexane
Acetone–H_2O
Thimet + some oxidized metabolites
Oxidized metabolites

Following this procedure, they presented residue data from applications of P^{32} Thimet on several vegetable crops. Soil applications to 24-foot rows was made by evenly distributing 300 ml. of emulsion containing 0.5 gram of toxicant. This was poured around the base of the plants with care to minimize surface contamination. The vegetable foliage was sampled randomly at 0, 1, 4, 8, 17, and 32 days after treatment. The residue information on the vegetables (beans, beets, cabbage, carrots, lettuce, and peas) was averaged, and 17 days after application less than 0.1 p.p.m. in Thimet equivalent was found in the plants. The data obtained on cotton residues with P^{32} Thimet are shown in Table III.

In a small-scale field test, alfalfa seed was coated with P^{32} Di-Syston (50% on activated charcoal) at the rate of 1 pound of toxicant per 25 pounds of seed. As shown in Table IV, the chloroform partitioning fraction (active metabolites) decreased rapidly and at 8 weeks represented only trace amounts, at which time cholinesterase assay with human plasma showed zero activity. When the results shown in Table IV, in which the radiotracer technique was used, are compared with those shown in Table II, in which the cholinesterase inhibition method was used, the similarity of results with the two methods of analysis is readily apparent.

From the residue data obtained on alfalfa from three methods of applying Thimet or Di-Syston at time of planting (Tables II and IV), it does not appear that there will be a residue problem at the time of first cutting or pasturing of the alfalfa at the rates used in these experiments. As previously discussed, residues in alfalfa dissipated in from 6 to 8 weeks, depending somewhat upon the rate of growth. In either case the alfalfa had attained a height of about 6 inches at the time of residue disappearance, and several weeks' further growth is needed before it is ready for the first harvest (114). Similarly, from the residue data obtained on cotton (17), as shown in Table III, it does not appear that there will be a problem of Thimet residue in the cotton seed, particularly when it is considered that the rate of material applied is from 4 to 8 times that which is being considered for commercial use.

TABLE IV

Residue of Chloroform-Soluble Di-Syston Metabolites in Alfalfa Grown after Seed Treatment with P^{32} Di-Syston at 1 Pound per 25 Pounds of Seed Using 50% Activated Charcoal

Weeks after treatment	P^{32} chloroform-soluble metabolites as p.p.m. Di-Syston in fresh leaves
2	121.5
3	22.0
4	12.1
5	1.14
6	0.50
7	0.21
8	0.007[a]

[a] Cholinesterase assay of this sample showed 0.0 p.p.m. (114).

When the persistence and magnitude of the residues on alfalfa and cotton are considered from the standpoint of using Thimet and Di-Syston on other crops, particularly vegetables with a short growing period from planting to harvest, the need for sound residue data is apparent. It appears from these data that seed treatment will constitute a negligible residue problem, and at this point it seems unlikely that granular formulations or soil-trenching applied at time of planting will constitute a major problem. Applications made on established plant stands by side dressing granular formulations or by some other suitable method of soil application, however, may be limited to crops which are several months from maturity or harvest at time of application.

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ISOTOPE DILUTION TECHNIQUES FOR THE DETERMINATION OF PESTICIDE RESIDUES

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CONTENTS

I. Introduction 184
  A. Scope 184
  B. Development of Concept 184
II. General Considerations 185
  A. Advantages 185
  B. Limitations 186
  C. Steps in an Isotope Dilution Analysis 187
  D. Calculation of Results 187
III. Isotope Dilution Analysis for 2,2-Dichloropropionate 191
  A. Method of Measuring Total Dalapon 191
  B. Choice of Solvents 192
  C. Method of Chloride Determination 193
  D. Method of Counting Radioactivity 194
  E. Preparation of 2,2-Dichloropropionic Acid-Cl^{36} 195
    1. Discussion 195
    2. Procedure 196
IV. Detailed Analytical Procedures 198
  A. Dalapon in Grapes 198
    1. Discussion 198
    2. Procedure 199
  B. Dalapon in Alfalfa 201
    1. Discussion 201
    2. Procedure 202
  C. Dalapon in Cottonseed 203
    1. Discussion 203
    2. Procedure 204
V. Conclusion 205
  References 206

I. Introduction

A. SCOPE

The term "isotope dilution analysis" may be applied to any method of chemical analysis which involves diluting the hunted material initially present in the sample with a precisely known quantity of a particular isotopic form of the same substance. Depending on the requirements of the analyst, this isotopic substance may be either an isotope of one of the elements, or a discrete chemical compound incorporating in its structure a specific isotope.

B. DEVELOPMENT OF CONCEPT

The basic concept of isotope dilution analysis is very nearly as old as our knowledge of isotopes themselves. Paneth (9) proposed the idea in his book published three decades ago.

Ussing (15) described an isotope dilution method for analysis of the amino acid content of proteins with the help of amino acids containing deuterium atoms, and thus became the first to use such a method with a stable isotope. Foster (2) and Rittenberg *et al.* (5,10) followed by using stable isotopes in the isotope dilution determination of the amino acid content of proteins.

Keston, Udenfriend, and Levy (6) appear to have been the first to use an isotope dilution procedure with a radioelement. By utilizing isotope dilution in combination with paper chromatography of the *N*-*p*-iodophenylsulfonyl-S^{35} derivatives of amino acids, they were able to estimate the derivatives quantitatively without having to rely on complete resolution and quantitative elution of the amino acid derivatives.

In general, isotope dilution methods have been pioneered and most vigorously developed in the field of biochemistry. This trend still continues, but indications have begun to appear that this very useful tool is being rediscovered by those working in other fields.

Isotope dilution procedures have already been described as a means of determining pesticide purity. Sorensen (12) proposed a method for the specific determination of 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, and 4-chloro-*o*-toloxyacetic acid using an isotope dilution procedure. Trenner *et al.* (14) have described an isotope dilution method for the determination of the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane using the deuterium-labeled compound.

To the best of our knowledge, however, no one has hitherto described the use of an isotope dilution technique as a method of pesticide residue analysis. In the hands of the authors, isotope dilution procedures have given very satisfactory results in the determination of residues of dalapon (2,2-dichloropropionic acid) in cottonseed, in grapes, and in alfalfa.

Although we have not yet applied similar methods to the determination of residues of other pesticides, there is every reason to believe that isotope dilution procedures would greatly simplify numerous problems of pesticide residue analysis. In the following discussion, we shall attempt to present enough theory to show how the concept of isotope dilution may successfully be applied to the analysis of almost any residue. At the same time, we shall cite examples from our own experience with 2,2-dichloropropionic acid to illustrate some of these principles.

II. General Considerations

A. ADVANTAGES

By now, most of us are familiar with the very important use of radioactive pesticides on a laboratory scale in order to determine their fate in crops, in the insect, in the soil, and so forth. The work of Metcalf *et al.* (8) nicely illustrates the use of a short-lived tracer in field evaluation of probable pesticide residue levels. However, such tracer procedures are often impractical for field experiments, for their use may necessitate handling relatively large quantities of costly and somewhat hazardous chemicals. This is particularly true with compounds prepared from long-lived radioisotopes. Isotope dilution procedures, on the other hand, involve the use of only very small amounts of isotopic compounds, for they require tagged materials to be added only to the analytical sample, rather than to the entire experimental plot. For pesticide residue work, isotope dilution procedures are distinctly more economical than simple tracer techniques; they may also be safer.

Furthermore, unlike other methods employing radioactive pesticides, isotope dilution procedures offer a means of studying residue levels in crops which have already been treated with nonradioactive pesticides. Hence, they are potentially useful for regulatory purposes. To the authors, this seems a particularly important point.

Additional advantages of the isotope dilution techniques follow from the fact that such schemes do not require quantitative recovery. The method, as will be seen shortly, automatically compensates for losses incurred because of adsorption on precipitates, volatilization, incomplete solvent extraction, and so on. Such a feature usually permits a wider choice of separation schemes for the purpose of achieving clean removal from interfering materials, including some which would be out of the question if the separation necessarily required every step to be free from partial loss of the residue. In addition, this wider choice can often result in the utilization of very simple separation schemes, thereby reducing the labor required for each determination. Because of the wider latitude in choice of separation schemes, the magnitude of the sample blanks observed can usually be made lower for isotope dilution methods than for the customary types of residue analysis.

Furthermore, if a radioactive diluent of sufficiently high specific activity is available, incorporation of isotope dilution procedures may lower the minimum useful limits of an existing analytical scheme markedly.

A large part of the published procedures utilizing the principle of isotope dilution have been gravimetric; and, like all gravimetric procedures, these have required a complete separation of the estimated compound from all other compounds. Consequently, they have been laborious. Contrary to this trend of the past, isotope dilution procedures need not be gravimetric determinations, and therefore they do not necessitate the isolation of a pure material any more than do colorimetric or titrimetric procedures. Only separation from materials interfering with the estimation of the residue by the particular method at hand is necessary. A realization of this fact should contribute considerably to the popularity of isotope dilution with the analyst.

B. LIMITATIONS

Isotope dilution analyses, like any other type, have their limitations as well as their potentialities.

Two of the limitations are a direct consequence of using radioactive compounds. First, an analyst cannot proceed with such a determination unless he is working in a laboratory equipped with at least a minimum of the instruments commonly used in the measurement of radioactivity in tracer work. Second, even if he has such equipment available, he must either prepare his own tagged compounds or else wait until they are commercially available. Imposing though these two obstacles may seem, the authors feel that they are more than offset by the economies of manpower utilization provided by the use of isotope dilution techniques.

A third limitation is that isotope dilution methods are accurate for analyses of tissues only if the added tagged material is adequately equilibrated with the untagged material prior to extraction. In the analysis of fleshy, easily minced tissues, this requirement should be easily met. Difficulty may be expected in work with samples consisting of many dry particles relatively impervious to the usual extracting media. If this fact is borne in mind at the outset of development of an analytical procedure, one may save himself much unnecessary effort.

C. STEPS IN AN ISOTOPE DILUTION ANALYSIS

Before the experimental results of an isotope dilution type of analysis can be interpreted, the analyst must do four things: (*1*) he must add an accurately known amount of isotopic substance to the total sample; (*2*) he must completely separate a part of the substance being estimated from interfering materials; (*3*) he must, in some way, determine the total quantity of this material which he has separated; and (*4*) he must measure the fraction of this estimated total which is due to the initially added isotopic substance.

The added isotope may be either a stable isotope or a radioactive one. However, the greater simplicity of quantitative microestimation of radioisotopes makes their use preferable for analyses in the microgram range, within which lie the majority of pesticide residue problems. For this reason, we shall limit our attention in the following discussion to the use of radioisotopes in isotope dilution techniques.

D. CALCULATION OF RESULTS

An exact set of equations applicable to isotope dilution work in general has been derived by Gest, Kamen, and Reiner (4). These equations take into consideration the fact that the tagged compound added in the dilution step has a slightly different molecular weight from the untagged compound. For the majority of cases in which labeled compounds have been prepared from radioelements, one may neglect these differences and still introduce an error too small to be of significance in residue work.

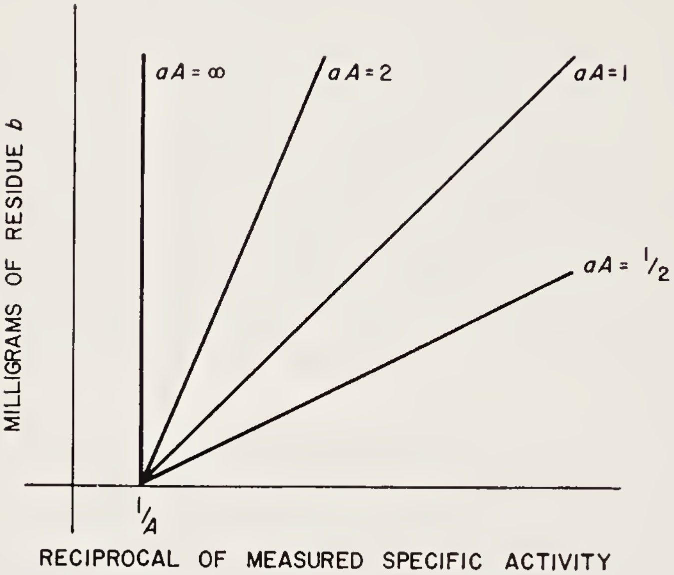

Fig. 1. Relationship between residue content of sample and reciprocal of specific activity after dilution.

In order to arrive at the basic simplified equation for isotope dilution analyses, let us assume that we had available a radioactive pesticide of specific activity of A counts per minute per milligram. Further, assume that we add a milligrams of this radioactive compound to the residue sample prior to extraction. Let b represent the number of milligrams of untagged pesticide initially present in the sample, and C represent the specific activity of the total pesticide in the sample after dilution with the radioactive compound.

Now, the total radioactivity added is aA counts per minute. By definition, the total number of counts per minute is $C(a + b)$. Inasmuch as all of the radioactivity has to arise from the added tagged pesticide,

$$C(a + b) = aA \tag{1}$$

Solving for b, we obtain:

$$b = a(A/C - 1) \tag{2}$$

Inasmuch as both a and A can be measured accurately without recourse to extraction and purification procedures, and inasmuch as C can be determined and will remain unchanged, regardless of losses during the isolation and purification process employed, equation (2) may be used to evaluate accurately the initial residue content of the sample.

A plot of a family of curves derivable from equation (2) is shown in Fig. 1. Usually, after an isotope dilution procedure has been developed for a particular residue problem, the specific activity, A, and the weight, a, of the tagged compound are held constant from one determination to another. Hence, a and A may be regarded as parameters. $1/C$ is measured for each sample individually, and b is the value sought. Figure 1 shows that for a given procedure, the residue found will be a linear function of the reciprocal of the specific activity of the sample after dilution and purification.

The slope of this straight line is numerically equal to aA, the radioactivity added to the sample. When a large amount of radioactivity is added to the residue sample, a unit increment in residue content, b, will result in only a very slight increase in the reciprocal of the specific activity, and the accuracy will be poor. As the value of aA becomes smaller, a unit increment in residue content results in a greater increase in the reciprocal of the specific activity, and the accuracy improves. Hence, except for the presence of counting errors, the accuracy should keep improving as the amount of radioactive diluent is made smaller and smaller.

In actual practice, the observed "background" counting rate sets a very definite limit on the advantages to be gained by reducing the quantity of tagged compound added. Because the pulses constituting the background count of any device for measuring radioactivity are not produced at a strictly uniform, constant rate, there is always a considerable degree of uncertainty regarding the magnitude of the background count at any given time. Therefore, even for inconveniently long counting periods, a very low specific activity for the counted sample will introduce rather large counting errors into $1/C$, and hence into the result of the analysis.

The relationship between probable counting error, counting time, and total observed count rate for a Geiger-Müller tube is shown in Fig. 2 (13).

The optimum amount of tagged diluent to add to a sample for residue analysis will vary somewhat with the accuracy with which the total amount of residue in the purified extract may be determined. It will also depend on the minimum acceptable accuracy of a given determination, on the prevailing background count rate, and on the maximum permissible counting time for a sample. In any event, the quantity added should be held to as low a value as other considerations will permit. For typical residue work, a scheme which will result in planchets counting at between one and two backgrounds above background will roughly approximate optimum conditions.

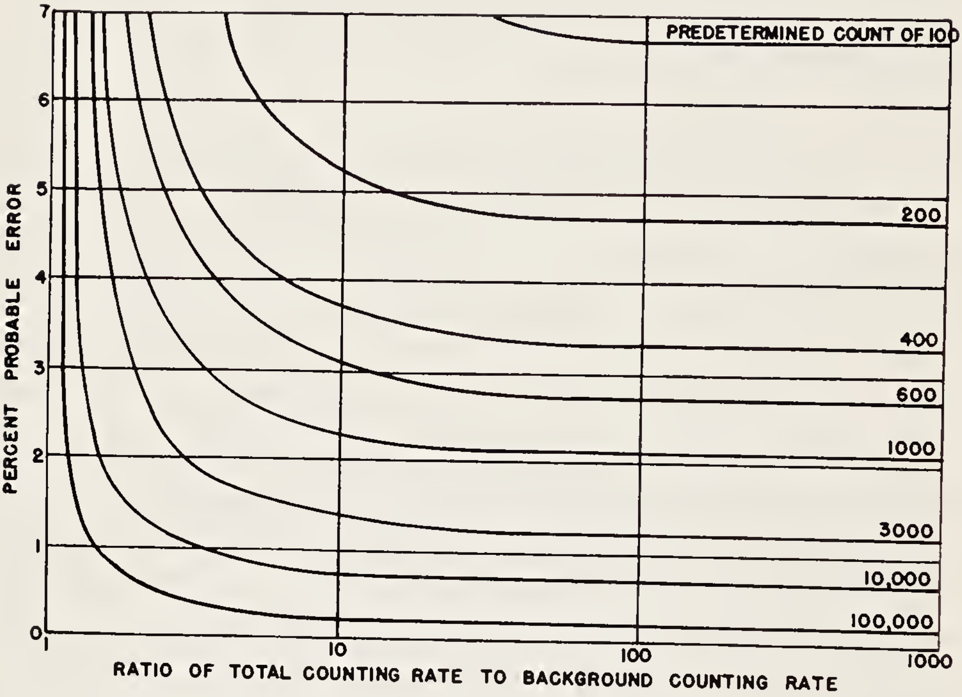

Fig. 2. Probable error vs. ratio of total counting rate to background counting rate, for various preset count totals (from *SC-18 Superscaler Operating and Maintenance Instructions*, p. 8, by permission of Tracerlab, Inc., Boston).

III. Isotope Dilution Analysis for 2,2-Dichloropropionate

The steps in the development of a typical isotope dilution procedure may be illustrated by our own experience in designing methods for analysis of dalapon (2,2-dichloropropionic acid) residues.

A. METHOD OF MEASURING TOTAL DALAPON

The first problem we had to solve was to find a means of estimating dalapon in the purified extracts in order that this information, along with the count rate, could be used to calculate the specific activity, *C*. Two possibilities occurred to us. The first was to convert the 2,2-dichloropropionate to pyruvate by hydrolysis and estimate the resulting pyruvate by one of the published colorimetric procedures. The other was to hydrolyze the 2,2-dichloropropionate and determine the resulting chloride.

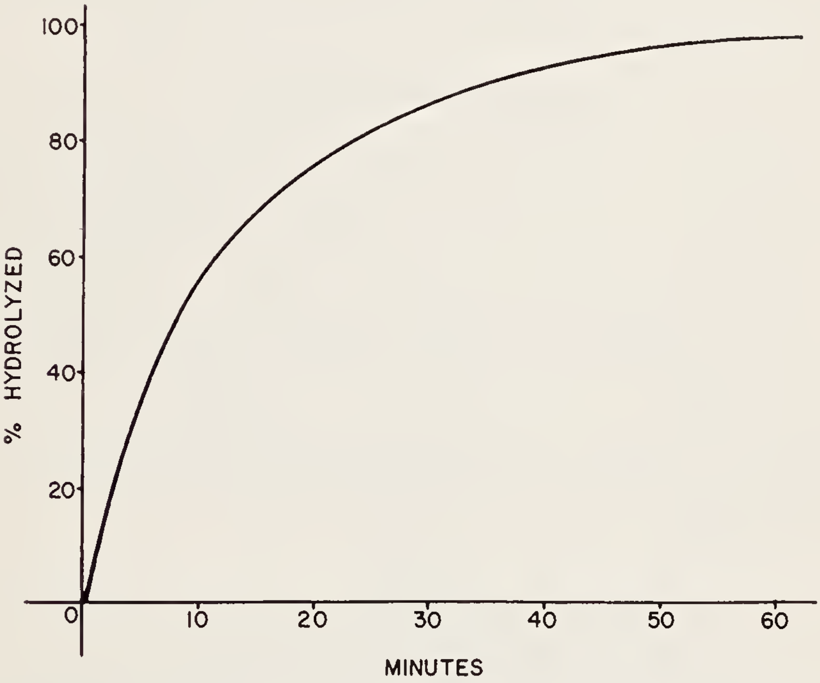

Fig. 3. Rate of hydrolysis of 2,2-dichloropropionate by 10% sodium carbonate at 120°C.

Early experience showed that certain crops contained large amounts of materials producing pyruvic acid during hydrolysis in the presence of alkali. Also, the indications were clear that a clean separation of 2,2-dichloropropionate from these interfering materials would be very difficult. Hence, we chose the other alternative: hydrolysis and estimation of the liberated chloride.

Next, we found that the chlorine atoms in the 2,2-dichloropropionic acid molecule were readily converted into chloride by heating with a 10% sodium carbonate solution at 120°C., a temperature easily reached in a laboratory autoclave or household pressure cooker. The rate data for this conversion are shown in Fig. 3. Hence, we felt confident that this conversion of dalapon to chloride was a sound basis for analysis.

B. CHOICE OF SOLVENTS

We were also confident that we could find a suitable solvent partition scheme for separation of dalapon from interfering inorganic chloride and from all halogenated organic compounds except carboxylic acids readily soluble in water and in a water-immiscible solvent. But before we could devise such a scheme, we would have to know more concerning the partition coefficients for 2,2-dichloropropionic acid between dilute mineral acids and a number of immiscible organic solvents.

Partition coefficients for 2,2-dichloropropionic acid between 1*N* hydrochloric acid and some of the solvents which we considered are shown in Table I.

TABLE I

Partition Coefficients for 2,2-Dichloropropionic Acid between 1*N* Hydrochloric Acid and Immiscible Solvents at 25°C.

Solvent	Partition coefficient
tert.-Amyl alcohol	30
n-Butyl alcohol	23
Ethyl ether	6.5
Methylene chloride	0.7
30–60° Petroleum ether	0.09

The two alcohols, although better solvents for 2,2-dichloropropionic acid than ethyl ether, were eliminated from further consideration after it was found that they were also good solvents for salts of the acid. Such a property would substantially hinder the transfer of the acid from a water-immiscible phase into a much smaller volume of aqueous base. Hence, ethyl ether, the best of the remaining solvents, was adopted for our use.

The exact details of the extraction and purification procedures varied from crop to crop. The goal was always to separate the 2,2-dichloropropionic acid from interfering materials and simultaneously to increase the concentration above that initially present in the sample extract. The requirements of counting and of halide determination made this latter feature particularly desirable.

C. METHOD OF CHLORIDE DETERMINATION

Trial runs of our simplest purification schemes showed that some of the purified extracts tended to form highly colored products on autoclaving with alkaline solution. Hence, we chose to use a potentiometric method of the chloride estimation, rather than a titrimetric procedure.

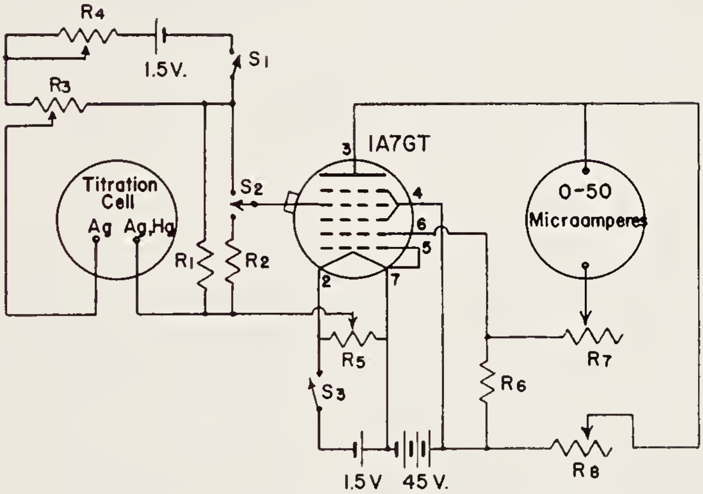

Fig. 4. Circuit diagram of potentiometer utilizing electronic millivoltmeter as a null indicator. R_1, R_2, 100,000 ohm, 1/2 watt; R_3, 1000 ohm Helipot; R_4, 10,000 ohm Helipot; R_5, 5,000 ohm Helipot; R_6, 2,000 ohm, 1/2 watt; R_7, 50,000 ohm volume control; R_8, 2,500 ohm semivariable. (Adjust for individual tube to bring meter reading on scale. When R_2 is connected to grid of tube through S_2, R_7 is at maximum value, and R_5 is near mid-point of range.)

Titration with $5 \times 10^{-4}\, N$ silver nitrate, using the silver and silver amalgam electrode pair described by Kirk (7), was chosen primarily for its simplicity and its adaptability to the microgram range. We have found that Kirk's electrode pair is thoroughly satisfactory, even with sample volumes of the order of 0.1 ml., provided that two precautions are observed: (*1*) a "known" sample should be titrated before an actual sample in order to condition the electrodes after they have stood idle for more than a few hours; and (*2*) special care must be taken not to employ a stirring apparatus which can accidentally brush against the amalgamated electrode during a titration.

For agitation during titration, we have employed a commercially available magnetic stirring device, in conjunction with stirring bars fashioned from one-quarter inch lengths of wire brads sealed into Pyrex glass capillary tubes.

As an indicating device for this electrode pair, we used a potentiometer combined with a vacuum tube millivoltmeter null indicator. The circuit diagram of this apparatus is presented in Fig. 4. The basic vacuum tube millivoltmeter is an adaptation of the circuit of Garman and Droz (3). In our hands, this potentiometer and electronic millivoltmeter combination has given excellent results, although a conventional potentiometer in combination with a good galvanometer should have been equally useful.

D. METHOD OF COUNTING RADIOACTIVITY

The exact details of the counting technique employed may be varied to fit the particular equipment available. We had at our disposal an automatic sample changer which accommodated planchets 26 mm. in diameter. There were also available for use with this sample changer aluminum disks and rings suitable for mounting 25 mm. diameter disks for counting.

We employed an adaptation of the procedure described by Calvin *et al.* (1). We found that 25 mm. disks of Shark Skin filter paper would nicely blot up 0.100 ml. of 10% sodium carbonate hydrolyzate. Hence, we adopted the procedure of placing 25 mm. disks of Shark Skin filter paper in the 26 mm. diameter nickel-plated dishes, pipetting onto them 0.100 ml. of hydrolyzate, and subsequently drying on the steam bath. When dry, the paper disks impregnated with sodium carbonate and sodium chloride are stripped off the dishes, mounted on the aluminum disks with the concentric mounting rings, placed in the sample changer, and counted with a mica end-window Geiger-Müller tube. Errors due to pipetting and mounting are minimized by counting several replicate dishes for each sample.

The mass of sodium carbonate (10 mg.) and paper (20 mg.) is so great in comparison to the maximum probable mass of 2,2-dichloropropionate present in 0.100 ml. of autoclaved carbonate solution that self-absorption may be neglected as a source of counting error. Also, because of the constancy of the technique of plating, the surface density of the plates has been held constant and no beta-ray absorption correction need be made here. These facts are verified by the experimental data presented in Fig. 5.

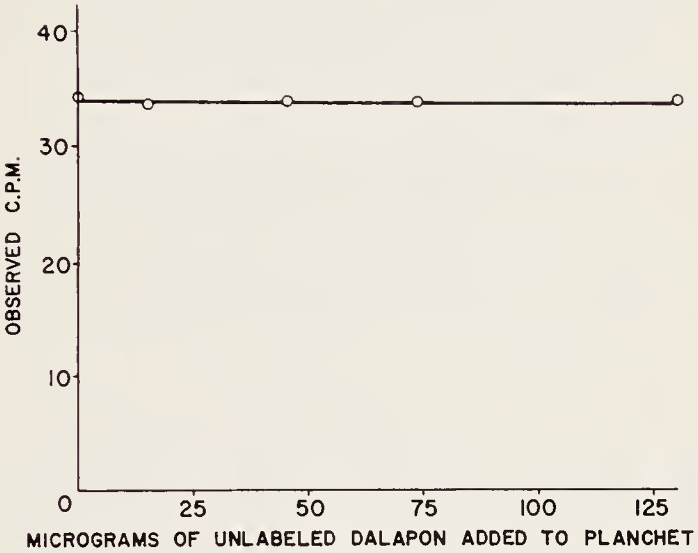

Fig. 5. Relation between count rate observed for a unit of tagged 2,2-dichloropropionate and the total weight of 2,2-dichloropropionate on the planchet. Sample deposited on 20 mg. paper disk with 10 mg. of sodium carbonate.

Beta-ray absorption by other extractives present in the autoclaved carbonate may be detectable if these extractives add any appreciable mass to that of the sodium carbonate and paper. Such additional absorption of radiation can result in an appreciable sample blank if measures are not taken to minimize it. As a matter of fact, this is probably an important source of the sample blank observed for the examples cited later in this chapter.

E. PREPARATION OF 2,2-DICHLOROPROPIONIC ACID-Cl^{36}

1. Discussion

The next matter to consider was the availability of labeled 2,2-dichloropropionic acid. Either a carbon-labeled or a chlorine-labeled product would have been satisfactory, although the use of a carbon-labeled product would have introduced a number of additional purification problems into the analytical procedures. Fortunately, synthesis of 2,2-dichloropropionic acid-Cl^{36} by means of an exchange reaction between 2,2-dichloropropionic acid and lithium chloride-Cl^{36} in acetone proved to be a relatively simple and successful process.

In this exchange reaction, the equilibrium constant has been found to be unity when determined in terms of inorganic and organic chlorine. Accordingly, one can prepare dalapon-Cl^{36} with a specific activity approaching that of the lithium chloride-Cl^{36} by a suitable adjustment of the stoichiometry.

The exchange reaction between lithium chloride-Cl^{36} and dalapon obeys a first-order rate law because the concentrations of the chemical species do not vary with time. The rate at 80°C. was negligible, whereas at 120°C. it was of such a value that the reaction could be allowed to proceed conveniently overnight without appreciable competition from side reactions.

2. Procedure

Carefully mix in aqueous solution equivalent quantities of hydrogen chloride-Cl^{36} and lithium carbonate. Evaporate to dryness on a steam bath to obtain a quantitative yield of the extremely deliquescent lithium chloride-Cl^{36}. This compound must be stored in a desiccator.

Seal in a Pyrex glass tube, or some suitable glass-lined pressure vessel, lithium chloride-Cl^{36} and 2,2-dichloropropionic acid along with sufficient purified acetone to make the reaction mixture homogeneous. This amounts to 1 ml. of acetone per 0.05 mg. of lithium chloride. Care must be taken to exclude moisture from the reactants, solvents, and pressure vessel. The acetone is purified by distillation from potassium permanganate as described by Vogel (16).

Heat the glass tube in an autoclave at 120°C. for 24 hours. Cool the vessel to room temperature and open it carefully. Transfer its contents to a distilling flask carrying a 16 cm. long Vigreux column, and distill off the acetone until only a few ml. remain. Care must again be exercised to exclude moisture from the contents of the flask. Triturate the residue of lithium chloride with three portions of alcohol-free anhydrous ether to remove most of the dalapon-Cl^{36}. Dissolve the lithium chloride remaining in a small volume of water, and extract with three equal-volume portions of alcohol-free ethyl ether.

Dry the ether extracts of the aqueous solution over anhydrous magnesium sulfate. Combine with the ether used for the trituration, and concentrate to a volume of less than 5 ml.

Transfer the concentrated ether and acetone solution of dalapon-Cl^{36} to vessel *A* in the apparatus illustrated in Fig. 6. Freeze the contents of tube *A* with liquid nitrogen, and immerse trap *B* in a liquid nitrogen bath. Evacuate the system to 10^{-2} mm. and exchange the liquid nitrogen cooling bath at tube *A* for a dry ice and acetone bath. By careful manipulation of the cooling bath at tube *A*, slowly distill the contents of this tube into trap *B*, continuing the pumping during the process. To insure complete transfer, warm tube *A* to

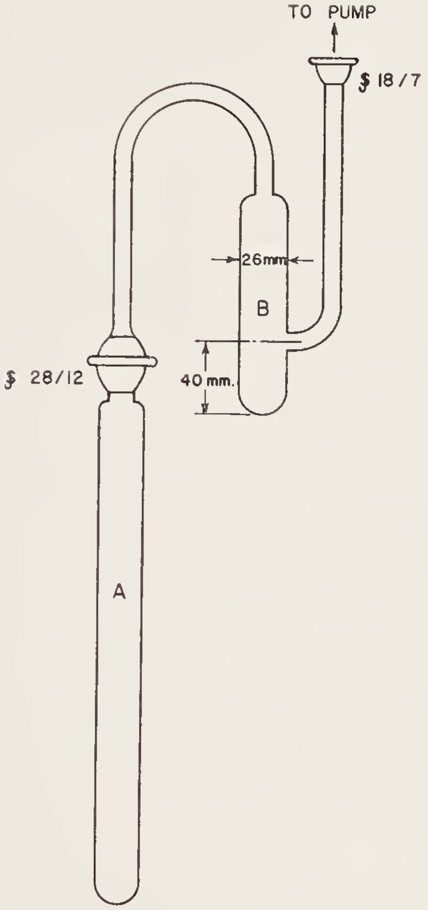

Fig. 6. Diagram of manifold employed in the synthesis of chlorine-tagged 2,2-dichloropropionic acid.

about 50°C., and continue the pumping for 15 mintes longer after the ether appears to have been removed to tube *B*. Take care to avoid warming tube *A* while it still contains visible solvent, since a flash distillation might occur and carry over lithium chloride-Cl^{36}, which may have come through the work-up scheme.

After the distillation is complete, vent the system with dry air. The ether-acetone solution of dalapon-Cl^{36} can be stored unchanged for many months in the cold, as long as water is omitted. The recovery of dalapon is high, 70–90%. Paper chromatography using *n*-butanol and 1.5*N* ammonia, 1:1 v/v, indicates the presence of only one compound, 2,2-dichloropropionic acid.

IV. Detailed Analytical Procedures

Having decided on a means of estimating total dalapon, a suitable method of preparing the tagged diluent, and a satisfactory means of measuring radioactivity, we needed only to work out a simple extraction and purification scheme for each crop. Recounting in detail all of the minute reasons for the choice of a particular extraction and purification plan would accomplish little, for many of them would be unique for one crop and one residue. Instead, we have chosen at this point to introduce the final analytical procedures adopted for the isotope dilution analysis of dalapon in three crops: grapes, alfalfa, and cottonseed.

These three examples illustrate clearly the way in which the concept of isotope dilution may be introduced into an analytical procedure, and at the same time they show the remarkable simplicity of purification schemes which may be used with isotope dilution techniques.

A. DALAPON IN GRAPES

1. Discussion

The scheme developed for the isotope dilution analysis of grapes for 2,2-dichloropropionate illustrates an analytical procedure suitable for a soft, easily minced material. Proteins, which would favor stable emulsion formation, are removed from the juice of the finely ground tissue by means of a phosphotungstic acid precipitation. The 2,2-dichloropropionic acid is extracted from the clarified filtrate with ethyl ether. A series of partitions between ether and aqueous buffers serves to concentrate the 2,2-dichloropropionate and separate it from interfering materials.

The recoveries found for varying amounts of dalapon added to 200 g. samples of grapes are presented in Table II. These data illustrate experimental values typical of an actual analysis, and demonstrate that the method is reliable even though simple.

TABLE II
Recovery of Untagged 2,2-Dichloropropionate from Grapes

Dalapon added, p.p.m.	Net counts/ min.	Mean vol. $5 \times 10^{-4}N$ $AgNO_3$, ml.	Total untagged dalapon found, p.p.m.	Total dalapon, less sample blank of 0.12 p.p.m., p.p.m.	Apparent recovery, %
0.0	67.3	0.32	0.08		
0.0	55.4	0.34	0.16		
0.0	50.9	0.58	0.45		
0.0	68.5	0.47	0.20		
0.0	66.6	0.29	0.06		
0.0	60.7	0.20	0.01		
0.0	63.9	0.26	0.05		
0.0	66.4	0.25	0.03		
0.0	60.4	0.21	0.02		
0.0	61.6	0.40	0.18		
0.41	63.1	0.80	0.53	0.41	100
0.41	61.0	0.72	0.48	0.36	88
0.83	72.5	1.50	0.97	0.85	102
0.83	68.4	1.39	0.94	0.82	99
2.5	74.0	3.59	2.50	2.4	96
2.5	75.0	3.68	2.53	2.4	96
8.3	72.7	10.85	8.06	7.9	95
8.3	76.4	11.90	8.41	8.3	100
17.8	66.1	20.40	16.9	16.8	95
17.8	63.7	20.10	17.2	17.1	96
				Mean	= 97%

2. Procedure

Place 200 g. of grapes in the jar of a Waring Blendor. Add 50 ml. of water and grind at top speed for about 5 minutes. Add 2.0 ml. of an acetone solution of 2,2-dichloropropionic acid-Cl^{36}. (Our solution contained 17.5 γ of 2,2-dichloropropionic acid in each milliliter and had a specific activity of 79.5 counts per minute per microgram of 2,2-dichloropropionic acid.) Blend for another 5 minutes. Add 55 ml. of 25% phosphotungstic acid $4N$ in sulfuric acid and 5 g. of filter aid. Blend for another 3 minutes. With the aid of a vacuum, filter off the pulp and precipitated protein on a paper filter coated with a layer of filter aid. Save the filtrate and discard the filter cake after sucking it as dry as possible.

Transfer the filtrate to a 500 ml. separatory funnel and shake out with two successive 150 ml. portions of ethyl ether. Combine the ether extracts and shake them out with one 20 ml. portion of saturated sodium bicarbonate solution. Acidify the bicarbonate extract to Congo red with $5N$ sulfuric acid, and saturate with anhydrous sodium sulfate. Shake out the saturated solution with two successive 25 ml. portions of ethyl ether. Combine these ether extracts, and shake out with 1.7 ml. of 10% sodium carbonate solution. Transfer the carbonate extract to a 10 ml. Erlenmeyer flask and cap with a 1 inch square piece of aluminum foil.

Place in an autoclave and heat at 120°C. for 1 hour. Cool to room temperature.

Plate out four 0.100 ml. portions on Shark Skin filter paper disks, dry, mount, and determine the radioactivity with a mica end-window Geiger-Müller tube (<2 mg./cm.^{2}) and scaler. Count for at least 1000 counts. Use the mean of the four counting times in calculating the count rate.

Determine the chloride content of duplicate 0.500 ml. portions of the carbonate solution by adding cautiously to each aliquot 0.30 ml. of $18N$ sulfuric acid and titrating with $5 \times 10^{-4}N$ silver nitrate, using the silver and silver amalgam microelectrodes in conjunction with a potentiometer. Use the mean of the two titrations for calculation of the residue level.

Determine the specific activity and concentration of 2,2-dichloropropionic acid in the diluent acetone stock solution employed by adding 5 drops of 10% sodium carbonate solution to 3.0 ml. of the acetone solution, evaporating to dryness, adding 2.0 ml. of 10% sodium carbonate, and autoclaving at 120°C. for 1 hour. Cool to room temperature, and dilute to exactly 5.0 ml. with 10% sodium carbonate. Titrate and count as for the analytical sample. Interpret the titration and count data in terms of the residue levels by the method of calculation already described (Sec. II.D.).

B. DALAPON IN ALFALFA

1. *Discussion*

The procedure employed for the analysis of alfalfa illustrates the use of hot 85% methanol as an extracting medium. This variation avoids the use of inorganic precipitants entirely and is a simple and

TABLE III

Recovery of Untagged 2,2-Dichloropropionate from Alfalfa

Dalapon added, p.p.m.	Net counts/ min.	Mean vol. $5 \times 10^{-4}N$ $AgNO_3$, ml.	Total untagged dalapon found, p.p.m.	Total untagged dalapon, less sample blank of 0.6 p.p.m., p.p.m.	Apparent recovery, %
0.0	37.0	0.35	0.86		
0.0	39.6	0.34	0.74		
0.0	60.2	0.41	0.49		
0.0	40.9	0.45	1.07		
0.0	45.6	0.28	0.40		
0.0	52.7	0.42	0.66		
0.0	33.6	0.25	0.58		
0.0	25.5	0.17	0.55		
0.0	60.4	0.38	0.43		
0.0	53.3	0.39	0.56		
1.3	24.9	0.45	2.0	1.4	108
1.3	43.1	0.71	1.8	1.2	93
5.2	21.2	0.98	5.9	5.3	102
5.2	43.5	1.93	5.6	5.0	96
10.5	39.2	3.08	10.3	9.7	92
10.5	52.0	4.09	10.3	9.7	92
20.9	39.3	5.89	20.1	19.5	93
20.9	56.2	8.27	19.7	19.1	91
52.4	55.9	20.20	49.1	48.5	93
52.4	40.6	16.18	54.1	53.5	102
				Mean =	96%

convenient procedure. Unfortunately, however, it is not universally applicable; some agricultural products—cottonseed meal, for example—contain methanol extractable materials which follow the dalapon through subsequent purification steps and obscure the end point of the subsequent potentiometric chloride titration.

The recoveries found for various quantities of dalapon added to 100 g. samples of alfalfa are presented in Table III. Increasing the sample size would probably improve the accuracy of the analyses, but a mean recovery of 96% is satisfactory for most residue work.

2. Procedure

Grind about 110 g. of fresh alfalfa in a meat grinder, exercising care to save the juice as well as the pulp.

Place 100 g. of the ground tissue in a 1 liter Erlenmeyer flask. Add 1.0 ml. of an acetone solution of 2,2-dichloropropionic acid-Cl^{36} (see Sec. III.E.2.) and 500 ml. of methanol to the contents of the flask and heat under reflux until most of the chlorophyll has obviously been extracted from the plant tissue. This should require about 15 minutes.

Decant the methanol extracts through a paper filter into a 1 liter Erlenmeyer flask. Add a saturated solution of sodium bicarbonate dropwise until enough is present to cause a drop of the methanol solution to produce a pH of 8 when added to a drop of distilled water.

Add boiling chips and distill off the greater part of the methanol at atmospheric pressure. Continue the distillation until the residue in the flask foams noticeably. At this point, transfer the flask and its contents to a steam bath in a fume hood and continue the evaporation until the volume of residue in the flask reaches approximately 30 ml.

Cool the flask and its contents to room temperature under the water tap. Acidify to a Congo red end point with 5*N* sulfuric acid. Transfer to a 60 ml. separatory funnel and extract with one 20 ml. portion of 30-60° petroleum ether. Discard the petroleum ether extract. Saturate the aqueous phase with reagent grade sodium sulfate, and shake out with two successive 20 ml. portions of ethyl ether, centrifuging if necessary, to obtain a clean separation of phases.

Combine the ether layers in a clean flask, and wash out the separatory funnel with water, using particular care to remove solution from the stopper of the funnel.

Replace the ether extracts in the separatory funnel and shake out with two 5 ml. portions of saturated sodium sulfate solution. Discard the sodium sulfate wash, and shake out the ether extract with one 1.7 ml. portion of 10% sodium carbonate. Separate the sodium carbonate phase, collecting it in a 10 ml. Erlenmeyer flask.

Cover the flask with a 1 inch square of clean aluminum foil, and heat it in an autoclave at 120°C. for 1 hour. Cool to room temperature.

Plate out 0.100 ml. aliquots for counting, titrate 0.500 ml. aliquots, and standardize the diluent stock solution as already described in the procedure for the analysis of grapes (Sec. IV.A.2). Calculate the residue levels by the method described in Section II.D.

C. DALAPON IN COTTONSEED

1. Discussion

The procedure utilized for the analysis of 2,2-dichloropropionic acid residues in cottonseed is considerably more lengthy than those applied to grapes and to alfalfa. Two factors contributed to the difficulty of developing a simple analytical procedure for use with cottonseed: (*1*) the product contains much rather gummy proteinaceous material not readily penetrated by water-immiscible organic solvents and (*2*) cottonseed contains rather large quantities of unidentified acidic substances which disturb the operation of the silver and silver amalgam electrode pair.

The extraction difficulty was overcome by the use of ether to remove lipide, followed by an aqueous acid extraction of the ether-insoluble residue. The aqueous acid effectively disperses the dry protein, thereby aiding the tagged diluent to reach equilibrium with residues adsorbed on or absorbed within the protein particles.

The interference with titrations was overcome by careful adjustment of the pH during solvent partition and by the use of a petroleum ether extraction midway in the purification scheme.

The performance observed for the analytical procedure to be described is indicated by the data in Table IV. The mean recovery for 50 g. samples was found to be 99%, whereas the lowest individual recovery was 85%, a value often considered satisfactory for residue analyses. Again, the accuracy of the individual determination could probably have been improved by utilizing a larger analytical sample.

TABLE IV
Recovery of Untagged 2,2-Dichloropropionate from Acala 4-42 Cottonseed

Dalapon acid added, p.p.m.	Total untagged dalapon acid found, p.p.m.	Total untagged dalapon found, less sample blank of 0.1 p.p.m., p.p.m.	Apparent recovery, %
0.0	0.05		
0.0	0.17		
0.0	0.08		
0.0	0.24		
0.0	0.20		
0.0	0.02		
0.0	0.03		
0.0	0.13		
0.0	0.27		
0.0	0.02		
1.0	1.3	1.2	120
1.0	0.97	0.87	87
2.0	2.2	2.1	105
2.0	2.3	2.2	110
5.44	5.4	5.3	98
5.44	5.5	5.4	99
10.88	9.3	9.2	85
10.88	11.5	11.4	105
21.76	18.6	18.5	85
21.76	20.0	19.9	92
		Mean =	99%

2. *Procedure*

Grind about 60 g. of cottonseed in a meat-grinder style food chopper. Weigh a 50 g. aliquot of the ground sample and mix intimately, using mortar and pestle, with 1 ml. of an aqueous stock solution of 2,2-dichloropropionic acid-Cl^{36} (see Sec. III.E.2). Permit the sample to stand for 1 hour or more.

Place in a Soxhlet extractor and extract the lipide with ethyl ether for 8 hours. Remove the insoluble residue from the extraction thimble and air-dry at room temperature.

Suspend the dried fat-free cottonseed meal in 500 ml. of water. Add 10 ml. of concentrated hydrochloric acid and 50 ml. of 25% phosphotungstic acid. Blend in a Waring Blendor for 5 minutes.

Let stand for 1 hour and filter with suction through a pad of filter aid, such as "Dicalite."

Shake out the filtrate with two successive 100 ml. portions of ethyl ether. Combine the ether extracts in a 250 ml. centrifuge bottle. Add 30 ml. of a saturated aqueous sodium bicarbonate solution. Stopper with a clean rubber stopper and shake thoroughly, releasing the evolved carbon dioxide as necessary. Cap the centrifuge bottle with a square of aluminum foil secured in place with a rubber band and centrifuge with a force of 810 times gravity for 20 minutes.

Remove the aqueous phase from beneath the ether phase with a 25 ml. pipet, being careful not to entrain any of the ether phase. Because of the presence of radioactive materials in the aqueous solution, the pipetting should be done with the help of a rubber bulb. We have found the device manufactured by Instrumentation Associates, New York, particularly helpful.

Transfer the aqueous phase to a 60 ml. separatory funnel and discard the ether phase. Acidify the aqueous solution to Congo red paper with 5*N* sulfuric acid, and then shake out with 10 ml. of petroleum ether. Discard the petroleum ether extract. Saturate the remaining aqueous phase with sodium sulfate and shake out with two successive 20 ml. portions of ethyl ether. Combine the ether extracts and wash with two successive 5 ml. portions of saturated sodium sulfate solution. Extract the washed ether solution by shaking with 1.7 ml. of 10% sodium carbonate solution.

Place with carbonate extract in a 10 ml. Erlenmeyer flask. Cover with a clean square of aluminum foil, autoclave at 120°C. for 1 hour, and cool to room temperature.

Plate out 0.100 ml. portions for counting, and titrate 0.500 ml. portions, as described in Section IV.A.2. Calculate the residue levels by the method described in Section II. D.

V. Conclusion

These three examples illustrate only a few of the many possible means which one might devise for separation of a pesticide residue from interfering substances, but they do show that such a separation is not necessarily laborious.

The authors hope that these examples of the use of isotope dilution procedures in pesticide residue analysis will stimulate others to adapt such methods to the solution of their own problems. We are convinced that there lies latent in such methodology the power to solve even the most perplexing residue analysis problem with minimum effort.

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WOOL DIGESTION AND MOTHPROOFING*

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CONTENTS

I. Introduction 208
II. The Keratin-Digesting Insects 209
  A. Biology 212
  B. Nutrition 214
III. Wool Structure and Chemical Composition 218
IV. Wool Digestion 221
  A. Ingestion and Passage of Wool through the Digestive Tract . . . 221
  B. Effect of Mechanical Damage on Digestibility of Wool 223
  C. Effect of Alkalinity on Digestibility of Wool 224
  D. Characteristics of the Proteolytic Enzymes Present 225
  E. The Role of Reducing Conditions 227
  F. Excretion 230
  G. Detoxication and Storage Excretion 233
  H. The Role of Microorganisms 237
V. Digestion of Silk, Spongin, and Chitin 237
VI. General Methods of Control 239
VII. Methods for Assessing Mothproofing Agents 240
VIII. Mothproofing Methods 242
  A. Fluorides 243
  B. Triphenyl Methanes 243
  C. Mitin FF 245
  D. Pentachlorophenol 245
  E. DDT 246
  F. Dieldrin 247
  G. Other Insecticides 247
  H. Modification of the Molecular Structure of Wool 247
IX. Action of Mothproofing Agents 250
  A. Fluorides 250
  B. Eulan CN (Lanoc CN) 251
  C. Mitin FF 252
  D. DDT 254
  E. Modification of Molecular Structure 256
References 257

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I. Introduction

The epidermal structures of vertebrates frequently contain a large amount of one or another of the many members of the keratin group of scleroproteins. Keratins as a class are characterized by the presence of 8–16% (21) of the sulfur-containing amino acid cystine, the higher the cystine content the more resistant the particular keratin being to biological degradation. Keratin of varying cystine content is the chief constituent of wool, hair, horns, hoofs, and feathers; it also occurs in mammalian skin, reptilian scales, tortoise shell, and so on. The chemical stability of the wool fiber is attributed partly to its morphological structure, but rather more to its high content of cystine (10–13%) through which numerous disulfide bridges are formed.

Insects are the only animals in which digestion of keratin has been demonstrated. The insects involved are the larvae of a small number of dermestid beetles, the larvae of a few moths belonging to the family Tineidae (and of one belonging to the Oecophoridae), and the Mallophaga or chewing lice of birds (196). An early report that certain birds of prey were capable of digesting feather keratin (172) was refuted shortly afterward (123), and a similar belief that snakes and lizards can digest the fur and feathers of their prey is unsupported by any evidence (197).

Keratin in its various forms has been a widespread and—certainly since the appearance of birds some 150 million years ago in Jurassic times—an increasingly abundant material on the earth's surface. The nests of birds and mammals must have provided through the ages a convenient source of keratins in the form of feathers and fur. It is, therefore, rather surprising that the keratins are nutritively useless to the vast majority of animals. The insects mentioned earlier—but not the vast majority of insect species—have evidently evolved some rather unusual digestive mechanisms to enable them to degrade and utilize such a valuable source of protein. This being so, one might expect that highly selective stomach poisons may be discovered which will be effective only against keratin digesters. This expectation has already been realized.

There is little doubt that, until comparatively recent times, the nests of birds and mammals, their dead carcasses, the faecal pellets of carnivorous species, and dead insects must have provided the normal breeding grounds for many of the species of economic importance as wool pests today (78–80,83,110,111,202–204).

It is difficult to arrive at a satisfactory estimate of the annual losses caused by keratin-digesting insects, although these are clearly very much greater than most people realize, as may be seen from the current United States estimate of 200–500 million dollars per annum (Table I). The actual amount of wool or hair eaten is probably the least important item. Far more serious is the damage to the appearance of the article caused by the eating of even a small fraction of the material. Then there is the cost of commercial mothproofing treatments, including the cold storage of furs, the expenditure by the housewife on remedies and her time for special cleaning operations. Our interest in the mechanism of digestion of keratin by insects and in methods of mothproofing fabrics is certainly well justified.

TABLE I
Some Estimates of Loss Caused by Fabric Pests
(Partly after Wälchli (186))

Annual loss	Based on	Ref.
10,000 tons wool	World	126
£1 million	England	26
1% or >1,000 tons actually eaten	World	186
£7–10 million	World	5
\$100–500 million through direct damage; \$25 million for protective measures	United States	106
D.M. 12,425,000	West Germany	75
\$200–500 million	United States	44,99
\$66 million by housewife on remedies; \$22 million on cold storage and insurance; plus many millions on commercial mothproofing and protection of materials	United States	70
Yen 15 million	Japan	207

II. The Keratin-Digesting Insects

A sharp distinction should be drawn between those insects which are capable of digesting keratin and those which are unable to digest it, but which will occasionally injure fabrics (141). Incidental, although at times important, damage may occur when these latter insects come into contact with and chew through the fabric whether or not it is a mothproofed woolen, a synthetic fabric, or even a fabric made of spun glass. Such insects include termites, crickets, silverfish, and even some dermestid larvae. Specifically, one may mention the hide beetle *Dermestes maculatus* Deg., *Dermestes frischii* Kugelann (and probably other *Dermestes* species), the larger cabinet beetle *Trogoderma versicolor* (Creutzer), and also *Trogoderma sternale* Jayne. These four species have been shown to be unable to digest keratin (197). One must suspect also that, together with other *Trogoderma* species, the cabinet beetle *Trogoderma ornatum* (Say) is similarly unable to digest keratin, although it has been recorded in birds' nests and in stuffed birds and other animals (80). The white-shouldered house moth *Endrosis lactella* (Schiff.), which has been recorded on various occasions as damaging fabrics, and the mottled grain moth (*Nemapogon* (=*Tinea*) *granella* (L.)) are also unable to digest keratin (197).

Insects known or suspected of being able to digest keratin include some 30 species of moth larvae, some 15 species of beetle larvae, and the bird-infesting Mallophaga numbering many hundreds of species. The Lepidoptera and Coleoptera involved are listed in Table II from which it will be seen that only seven species (asterisked) of each order are recognized as fabric pests of any importance. Species which really belong naturally to the genera *Tineola, Tinea, Trichophaga, Amydria* (all of the subfamily Tineinae of Hinton (82)) *Hofmannophila, Attagenus, Anthrenus,* and *Anthrenocerus* are all almost certainly able to digest keratin. Of these there is little doubt that, when all factors are taken into consideration, *Tineola bisselliella** must be regarded as the most important single species. Excellent photographs of a number of these pest species are published by Wälchli (186). Many of the remaining species listed are probably capable of digesting keratin, although this has not been shown experimentally. Furthermore, they seldom or never damage fabrics, many of them being recorded only from outdoors and in such situations as bird or mammal nests or lairs. *Amydria* (=*Tinea*) *vastella* is an interesting species which bores in the horns of various ruminants in Africa. It may cause considerable damage to piles of horns from slaughtered animals and has been reported attacking horns of the living beast (24), although this requires confirmation (177). The scarabaeid *Deltochilum* has been included since the larval food appears to be either the feathers or fur comprising the inner mass of the "egg ball" (87). It is, of course, possible that microorganisms are involved here in keratin digestion.

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* As used subsequently in this chapter, *Tineola* refers to this species.

TABLE II
Lepidoptera and Coleoptera Known or Suspected of Being Able to Digest Keratin (80,83,87,110)
(Important fabric pests marked with asterisk. Nomenclature that of Hinton (80,83) where appropriate)

Lepidoptera	Coleoptera
Tineidae	_Dermestidae_
*_Tineola bisselliella_ (Hummel)	*_Attagenus piceus_ (Oliv.)
*_T. furciferella_ Zagulyayev	*_A. pellio_ (L.)
*_Tinea pellionella_ (L.)	_A. gloriosae_ (F.)
*_T. columbariella_ Wocke	_A. lobatus_ Rosenhauer
T. flavescentella Haworth	_A. schäfferi_ (Herbst)
T. metonella Pierce & Metcalf	_A. elongatulus_ Casey
T. fuscipunctella Haw.	_A. nigripes_ Casey
*_Trichophaga tapetzella_ (L.)	*_Anthrenus verbasci_ (L.)
*_T. percna_ Corbet & Tams	*_An. vorax_ Waterh.
T. abruptella (Woll.)	*_An. pimpinellae_ F.
Phereoeca walsinghami (Busch)	*_An. scrophulariae_ (L.)
P. uterella (Walsingham)	_An. fuscus_ Oliv.
P. allutella (Rebel)	_An. museorum_ (L.)
Amydria vastella (Zell.)	*_Anthrenocerus australis_ (Hope)
Monopis rusticella (Clerck)	_Scarabaeidae_
M. ferruginella (Hübner)	
M. monachella (Hübner)	_Deltochilum gibbosum_ (F.)
M. crocicapitella (Clemens)	
M. dicycla (Meyrick)	
M. ethelella (Newman)	
M. pseudagyrta (Meyrick)	
M. trimaculella (Snellen)	
M. weaverella (Scott)	
Accedes ganomella (Treitschke)	
A. semifulvella (Haworth)	
*_A. pallescentella_ (Stainton)	
A. fuscipunctella (Haworth)	
Praecedes thecophora (Walsingham)	
Scleroplasta liberiella (Zeller)	
Tenaga inquisitrix (Meyr.)	
Oecophoridae	
*_Hofmannophila pseudospretella_ (Staint.)	

The relative importance of the species asterisked in Table II varies according to climate and country, although most are cosmopolitan. According to Ferguson (44), in the United States east of the Rocky Mountains the bulk of the damage in the southern states is due to the clothes moth *Tineola,* whereas in the northern states carpet beetles, mainly *A. piceus* but also *An. scrophulariae, An. verbasci,* and *An. vorax** in that order, are more important. He suggests that *Tineola* may be adversely affected by the low winter humidity of heated houses in the north, although other evidence indicates that it will develop under dry conditions (see p. 217). On the other hand, not only is *A. piceus* more resistant to cold, but also its eggs are susceptible to fungal attack at high humidities (68). *An. verbasci* eggs are also susceptible to high humidity (92). In Europe the distribution is reversed, carpet beetles playing a less important part in the north than in middle or southern Europe, although the species proportions vary over this range. The differences may be related to different winter temperatures in houses in the two regions. Of the moths *T. bisselliella* is more important in northern and middle Europe and *T. pellionella* in the south (186). The occurrence of some species—e.g., *H. pseudospretella,* which requires a very humid environment (201)—is strictly limited by physical factors, although, under suitable conditions, they may cause serious damage. Following the development of methods for identifying insect damage to fabrics (141,210), it has been reported that more than half is caused by carpet beetles (187,212).

A. BIOLOGY

An important point to stress is that none of the adults feed on, or damage, fabrics; this activity is confined entirely to the larval stage. Some adults (e.g., *Tineola*) have very rudimentary mouthparts and take no nourishment whatever. Others (e.g., the dermestid beetles) feed on such materials as pollen and nectar. Although most species can lay eggs without access to food or water, egg production is generally far greater when these are available (80,88). In *Tineola,* mating takes place soon after the adult emerges, the female producing a sex odor which attracts the male and induces courtship behavior (159). Oviposition occurs within a day or so, often near the site of infestation, for the females do not fly readily. Most *Tineola* seen flying are males. Dermestid adults are not as restricted in their habits and occur regularly on various flowers as well as indoors.

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* There is some evidence that this is a synonym of *An. flavipes* Le Conte (16).

Many papers have been published on the general biology of a number of the more important keratin-digesting insects (64,68,69, 77,78,80,83,88,92,151,177–179), although there are still surprising gaps in our knowledge. Since a good deal of the data tends to conflict over details of the life cycle, an attempt has been made in Table III to present a composite summary from the literature rather than to quote from individual sources. As in other insects, fecundity and rate of development are greatly influenced by adequacy of diet, by temperature, and by humidity. When one or more of these is sub-optimum, the life cycle can be extended enormously, and, particularly when the diet is poor, the number of larval moults may be greatly increased. Thus in *Tineola* there were 5 instars when the average developmental period was 97 days, 15 were observed for development averaging 349 days, and the maximum number of instars observed was 40 (179). The ability of dermestid larvae to survive long periods without food is truly remarkable and is probably due to their large fat body reserves. For example, *Anthrenocerus australis* larvae have lived without food up to 230 days at 27°C. and 75% R.H. (97).

TABLE III
Life Cycle Details of Four Important Fabric Pests

	T. bisselliella	*A. piceus*	*An. verbasci*	*An. vorax*
Number of moults	5–40	6–20	5–13	6–29
Number of eggs per female	80–100	40–100	20–100	40–100
Length of life cycle				
In nature	3 mo.–3 yr.	1–3 yr.	1 yr.	6–12 mo.
Optimum laboratory conditions	5–7 wk.	6–7 mo.	8–12 mo.	6–8 wk.
Optimum temperature, °C.	25	30–35	28–30	30–35
Optimum R.H., %	75	low	30–40	30–40

It can be seen from Table III that, although the average number of eggs per female does not vary greatly, the length of life cycle is much shorter (and hence the capacity for rapid increase much greater) for *Tineola* and *An. vorax* than for *A. piceus* or *An. verbasci*. Calculations showing prodigious numbers of progeny arising from a single female insect in the course of a year are often meaningless, although possibly less so with wool-digesting insects than in many other instances, for an infestation or stored woolens may pass unnoticed by man and by natural enemies for such a period. Assuming that female *Tineola* each lay 100 eggs of which 50% develop to moths and of these a third are females, Titschack (177) calculated that in the fourth generation (about a year) the progeny of a single female numbers 470,000 larvae which eat between 21.5 and 46.5 kg. of wool. If the more accurate assumption is made that 50% of the progeny are females, the number of fourth generation larvae is 1,562,500 (205).

As one might perhaps predict from their differing geographical distribution, different species are very differently affected by temperature. An example of the influence of temperature on food consumption is shown in Table IV. Maximum food intake occurred at 32°C. with *An. vorax* and *A. piceus* and at 28°C. with *An. verbasci.* The last-named species does not tolerate higher temperatures at all well. The greater attack by *An. vorax* than *A. piceus* at the higher temperature is also well established. *Tineola* can survive temperatures under 0°C. for long periods and cause considerable damage between 10 and 15°C., whereas Dermestids cease feeding at the lower temperatures and some species (e.g., *An. vorax*) are reported to succumb at temperatures below 10°C. (211). However, another report states that larvae and adults of this species can survive long winter periods when day temperatures are as low as +2°C. to −2°C. (78). Cold adaptation may well play a part in explaining these divergent results.

TABLE IV
Influence of Temperature on Weight of Food Eaten by Dermestid Larvae
(After Wälchli & Zinkernagel (187))

	20°C.	24°C.	28°C.	32°C.
		(mg. per 10 larvae)		
A. piceus	19	24	73	140
An. verbasci	22	39	60	46
An. vorax	23	28	137	224

B. NUTRITION

In spite of the close association of wool-digesting insects with sources of wool, hair, or feathers, their basic nutritional requirements are very similar to those of other insects. It has been known for many years (177) that keratin is not a necessary constituent of their diet. In fact, growth is much slower and mortality much higher on wool, hair, or feathers than on various stored products which are available for infestation, such as fish or meat meal, casein, and whole and ground grains of various kinds. The fact that *Tineola* larvae seldom infest these products is presumably due to the behavior of the ovipositing female. Dermestid larvae are less restricted in their food habits, for they attack a rather wider range of materials, particularly grains and grain products as well as dead insects and various materials of animal origin in addition to the hair.

Although larvae of keratin-digesting insects will commence to eat and may cause some damage to thoroughly clean wool on which eggs have been laid, little development occurs and they do not survive for long. Clearly keratin alone is not an adequate diet. It becomes more adequate if stained with urine or sweat or contaminated in various other ways (53,181). Even as a source of essential amino acids, clean wool presents a very unbalanced diet, being poor in tryptophane, methionine, histidine, and lysine (53) and very rich in cystine, which is seldom if ever required by insects. The preferential attack which commonly occurs on soiled portions of woolen fabrics will certainly lead to a more balanced diet. Thus, in addition to salts, many of the water-soluble B vitamins are present in urine, and the B vitamins thiamine, riboflavin, nicotinic acid, and pantothenic acid are present in sweat. The concentration of vitamins in these sources of contamination is low in relation to the requirements of *Tineola.*, but growth factors will gradually accumulate in certain parts of unwashed garments (53). Emphasis is placed on the vitamin B complex, since other vitamins are not dietary requirements of insects. All insects do, however, require a suitable sterol, such as cholesterol.

These requirements explain the early reports of growth stimulation in *Tineola* following the addition of yeast to the diet (18,28). It was not long before part or all of the B complex was recognized to be necessary for growth (30). The five factors of the B complex (thiamine, riboflavin, nicotinic acid, pyridoxine, and pantothenic acid) which most insects seem to require are also of importance in the nutrition of *Tineola,* although only nicotinic acid (most important) and pantothenic acid were shown to be indispensable (53). In the absence of any or all of the other B vitamins mentioned and, to a lesser extent, of biotin or choline, growth was slowed down, an observation reminiscent of the nutritional studies with the beetles *Lasioderma* and *Sitodrepa,* which are partially independent of B vitamins by virtue of the intracellular symbiotic yeasts which they harbor (52,142). Nitrogen fixation *in vitro* by *Azotobacter* from the fat body of clothes moth larvae has been claimed (143), although symbionts capable of synthesizing vitamins have not been demonstrated in *Tineola* or in any other Lepidoptera. Furthermore, *Tineola* does not harbor a rich flora of microorganisms in the gut (29). It is interesting to note that riboflavin occurs in the malpighian tubules of *Tineola* larvae fed on a diet free of riboflavin (25), and larvae appear to be relatively insensitive to a dietary deficiency in this factor (53).

Tineola larvae will grow rapidly and successfully on a diet lacking in both carbohydrate and fat, the only requirements being a suitable protein, B vitamins, cholesterol, and salts (30,53). It has been suggested that, since thiamine is involved as a cofactor in the pyruvic acid stage of carbohydrate metabolism and in the metabolism of glycerides, the insensitivity to dietary thiamine may be related to the insensitivity to dietary carbohydrates and fats. The fact that growth is benefited by thiamine may be due to its role in catalyzing the deaminated residues of some of the amino acids through the pyruvic acid stage (83). Although carbohydrates and fats are not essential dietary constituents, both occur abundantly and in a typical fashion in the larval tissues and extreme caution is needed before any claim is made that there are fundamental differences in the intermediary metabolism of carbohydrates and fats in *Tineola* larvae. It is far more likely that the larvae are either able to synthesize various B vitamins or that these are supplied by gut or symbiotic microorganisms.

It is interesting to calculate the coefficients of digestibility

$$\frac{\text{Dry wt. food consumed} - \text{dry wt. excrement}}{\text{Dry wt. food consumed}} \times 100$$

of wool from data presented for other purposes. For *Tineola* this averages about 44% (178,179); for *A. pellio,* 57% (23); and for *An. vorax,* 45% (187). When yeast is added to the fabric, resulting in a doubled food consumption by *An. vorax,* the coefficient of digestibility remains about the same, although it varies somewhat with fabric type—e.g., army uniform 44%; flannel, 52% (187). Some typical values for other insects include 24% for the silkworm larva, 36% for *Aglais urticae* (L.), 46% for the mealworm *Tenebrio molitor* L., 49% for the armyworm *Prodenia eridania* (Cram.) (182), and 79% for the silverfish (98). The value for the dairy cow for dried grass is 72% (182). The low values for the phytophagous insects reflect their inability to digest cellulose, and similar low values for the wool-digesting insects their need to excrete the excess nitrogen and sulfur in their highly proteinaceous diet. Thus, from the figure of 25% for the wool residues in *Tineola* excreta (144) it follows that 86% of the wool swallowed is digested, 44% is incorporated into larval tissues or lost as carbon dioxide and water, and 42% is used as a vehicle for nitrogen (urates and urea) and sulfur excretion.

A striking feature of larvae of *Tineola* and some other Tineids is their ability to develop under very dry conditions, an ability dependent upon a capacity to metabolize most of their water requirements (15). Thus, *Tineola* developed on feathers with a moisture content of about 6% in an atmosphere of 20% R.H., although larvae grew more rapidly and the mortality was lower at 75% R.H. on feathers having a moisture content of approximately 12% (69). Only in very dry air do *Tineola* larvae lose water more rapidly than they produce it by metabolizing food or reserves (128).

It is thus possible to list six factors which go far to explain the unusual food habits of *Tineola* larvae: (*1*) the behavior and oviposition responses of the female, which result in eggs being laid on or near keratin rather than on various more nutritious stored products; (*2*) the ability of the larvae to digest keratin; (*3*) their relative insensitivity to the absence of certain B vitamins; (*4*) their indifference to dietary carbohydrates; (*5*) their indifference to dietary fat; and (*6*) their ability to grow under very dry conditions.

Far less is known of the nutrition of wool-digesting Dermestids than of *Tineola*, although quite extensive information is available on the hide beetle *Dermestes maculatus* (= *vulpinus*) which, like other *Dermestes* species, is unable to digest wool. *D. maculatus* requires five of the B vitamins (riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and folic acid), and growth is slow in the absence of three more (thiamine, choline, and biotin), although small pupae are eventually produced. Inositol and *p*-aminobenzoic acid are not required, and large quantities of carnitine (vitamin B_T) are synthesized by the larvae (50,51). The presence of a suitable sterol is also essential (59). Active dietary sterols include cholesterol, cholesteryl acetate, and 7-dehydrocholesterol. *D. maculatus* differs from other insects so far studied in that it does not grow on ergosterol or sitosterol, the main sterols of microorganisms and of higher plants, respectively. The insects which grow on all three sterols feed on foods of vegetable and animal origin and on yeasts—unlike *D. maculatus*, which feeds exclusively on dry material of animal origin such as hides, bacon, and carcasses in which cholesterol is the main sterol (49,54). *D. maculatus* has the ability to produce much of its water requirements metabolically; it produces at least 66% when maintained on a yeast-fructose-cholesterol diet at 30% R.H. (52).

Parallel but less extensive studies have been carried out with *A. piceus* larvae which appear to require the same B vitamins (133,134), will grow on the same sterols (119,120), and require the ten amino acids in their diet which are essential to the rat (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine) (134). Dietary fat is not required by *An. vorax*, and larvae of this species cannot digest starch grains (149).

III. Wool Structure and Chemical Composition

A little should be known about wool structure and composition in order to understand the significance of the studies on wool digestion. The morphology of the wool fiber is extremely complex, and some of its finer features are still in dispute. The surface layer or cuticle of the fiber is composed of overlapping cells or scales which form a continuous tube around the central core or cortex (Fig. 1A,B,C). Lying underneath the scales and enveloping the cortex there is a very thin layer, the subcuticle. The scales which together form the cylindrical cuticle each consist of three layers (Fig. 1C). Within the cuticle and forming more than 90% of the fiber mass is the cortex, composed of long thin cells with pointed ends, closely packed and possibly cemented together (Fig. 1B,C). These cells in Merino wool are of two types (ortho, or soft, and para, or hard), each type being restricted to its own hemicylinder. This composite structure is responsible for the crimp, the hard hemicylinder always lying toward the inside of the crimp curve. In types of wool where the distribution of the ortho and para cells is radial instead of bilateral there is no crimp (56). Under the electron microscope at 20,000 × magnification the cortical cells can be seen to contain many fibrils embedded in a matrix, and at 100,000 × these fibrils are resolved into bundles of finer protofibrils (Fig. 1D). Each protofibril is believed to consist of a chain of protein macromolecules, each macromolecule containing some 4,000 amino acid units. About 19 different amino acids are involved, each with a different size and shape. These amino acid units are thought to be joined to form chains 400–500 units long, variations in the numbers and arrangement of the amino acids producing variations in the properties of the resultant protein. It is thought that each chain of amino acids is wound into a spiral and that the spirals in turn are wound together like the strands of a cable. The macromolecules which form the protofibrils probably contain a number of these cables lying parallel to each other and to the length of the fiber.

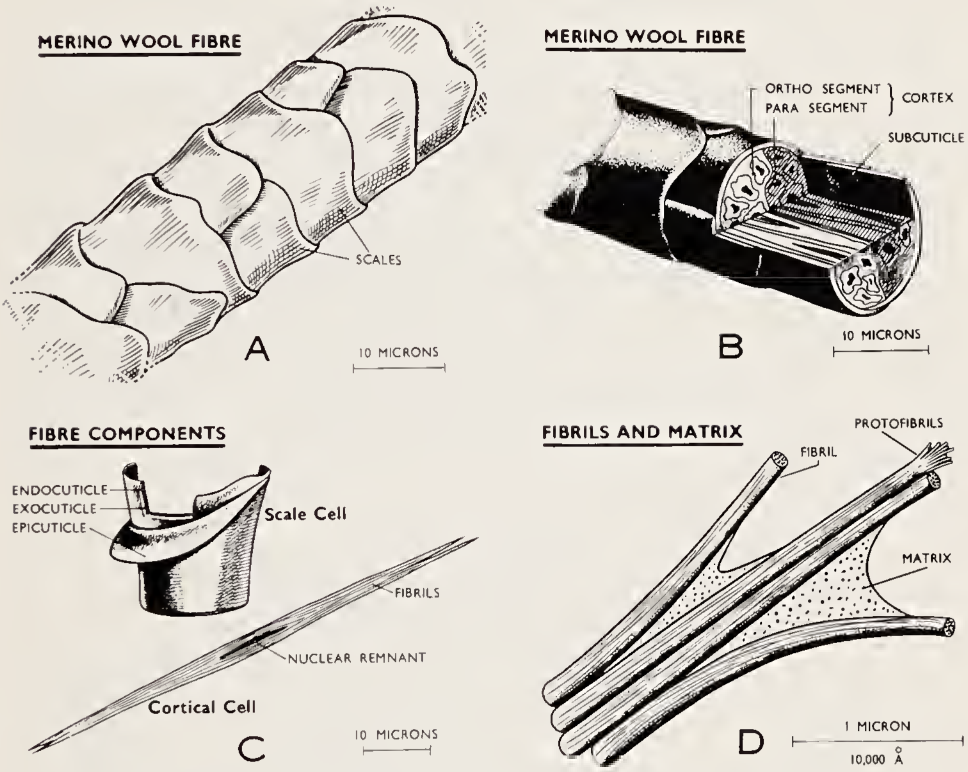

MERINO WOOL FIBRE
SCALES
A
10 MICRONS
MERINO WOOL FIBRE
ORTHO SEGMENT
PARA SEGMENT
CORTEX
SUBCUTICLE
B
10 MICRONS
FIBRE COMPONENTS
ENDOCUTICLE
EXOCUTICLE
EPICUTICLE
Scale Cell
FIBRILS
NUCLEAR REMNANT
Cortical Cell
C
10 MICRONS
FIBRILS AND MATRIX
FIBRIL
PROTOFIBRILS
MATRIX
D
1 MICRON
10,000 Å

Fig. 1. Structure of the Merino wool fiber (from Fraser (55)).

Coarse wool and hair contain within the cortex a central core or medulla which, unlike the scales and cortex, is readily digested by enzymes (41).

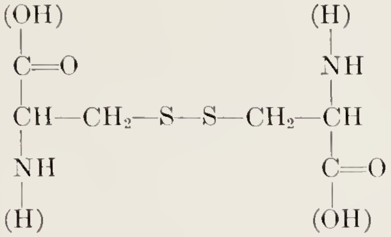
In addition to protein, which forms the main bulk, lipoids, sterols, and minerals are present in wool as it occurs in nature. However, most of these can be easily extracted, and wool can be considered as being almost entirely made up of protein with the average amino acid composition shown in Table V. The keratin group of proteins is characterized chemically by a high sulfur content, found mainly in the amino acid cystine, although a small amount of methionine is also present. Hair contains about 16% cystine; wool, 10–12%; feathers, 9–10%; and horn, 7% (21). There is some variation in amino acid composition in different wool samples due to the varying nutrition of the sheep and to several types of biological and mechanical degradation, such as weathering and exposure to light. The cystine content is smaller in the tip portion of wool than near the skin, since the former has been exposed longer to degradative influences. It is also less after commercial scouring (31). In the wool root there are many thiol groups which have not yet become oxidized to form disulfide bonds of cystine.

TABLE V
Average Amino Acid Composition of Wool
(After Harris & Brown (71))

	Per cent		Per cent
Alanine	4.4	Lysine	3.3
Arginine	10.4	Methionine	0.7
Aspartic acid	7.3	Phenylalanine	3.8
Cystine	12.7	Proline	6.8
Glutamic acid	15.3	Serine	9.4
Glycine	6.5	Threonine	6.8
Histidine	0.7	Tryptophane	0.7
Hydroxylysine	0.1		
Leucine Isoleucine	11.3	Tyrosine	4.7
		Valine	4.7

Keratin is relatively resistant to any chemical attack not involving the disulfide bond of cystine; this bond, however, is susceptible to reduction and may also be affected by oxidation and hydrolysis.

Since cystine is a dicarboxylic diamino acid, it can become part of adjacent polypeptide chains to form a disulfide cross link



$$\begin{array}{ccccccccccc}
\mathrm{(OH)} & & & & & & & & & & \mathrm{(H)} \\
| & & & & & & & & & & | \\
\mathrm{C{=}O} & & & & & & & & & & \mathrm{NH} \\
| & & & & & & & & & & | \\
\mathrm{CH} & - & \mathrm{CH_2} & - & \mathrm{S} & - & \mathrm{S} & - & \mathrm{CH_2} & - & \mathrm{CH} \\
| & & & & & & & & & & | \\
\mathrm{NH} & & & & & & & & & & \mathrm{C{=}O} \\
| & & & & & & & & & & | \\
\mathrm{(H)} & & & & & & & & & & \mathrm{(OH)}
\end{array}$$

There is good evidence (4) that most of the cystine is involved in such linkages, producing a stable three-dimensional lattice. The amino acid proline, which causes folding of the polypeptide chain, is also thought to confer resistance to enzyme attack (109).

It is not surprising that the various morphological components of the wool fiber have different compositions. The medulla of coarse wool and hair contains less cystine than the fiber as a whole (19), and this may explain its greater susceptibility to enzyme attack. The orthocortex and paracortex differ in cystine, proline and dicarboxylic amino acids (57). A higher proportion of cystine (60) and proline (109) in the scales than elsewhere has been claimed, but it must be remembered that these consist of three layers of differing composition. The epicuticle may or may not be protein but, instead, may contain polysaccharide and lipoid (4). It is easily damaged mechanically. Enzyme attack on scales first removes the exocuticle, leaving the more resistant endocuticle (130), so the composition of these two layers clearly differs. The subcuticle also differs in composition, for it probably contains no disulfide cross links. Instead it appears to contain links of a type (involving tyrosine) absent from the rest of the wool fiber (2). It is a serious barrier to the passage of anions (3).

In the intact fiber, therefore, there are not only important mechanical barriers to the penetration of digestive juices but also various linkages in the keratin molecule which confer relatively high resistance to enzyme attack.

IV. Wool Digestion

A. INGESTION AND PASSAGE OF WOOL THROUGH THE DIGESTIVE TRACT

Wool, as used in the textile industry, varies in diameter from 18 to 40 μ, although horsehair may range up to 250 μ. The maximum distention of the mandibles of young *Tineola* larvae is 90 μ according to Reumuth (151) who has studied feeding behavior. The fine fiber is seized by the maxillae, fed progressively into the pharynx, and bitten off into roughly equal lengths averaging 70–150 μ (40–120 μ in dermestid larvae (192)) depending on the larval instar (36); coarser fibers are attacked from the side and irregular fragments swallowed. In a thorough mixture of wool and synthetic fibers both are attacked seemingly without preference, although, if pure threads of each are mixed, the synthetic thread tends to be avoided.

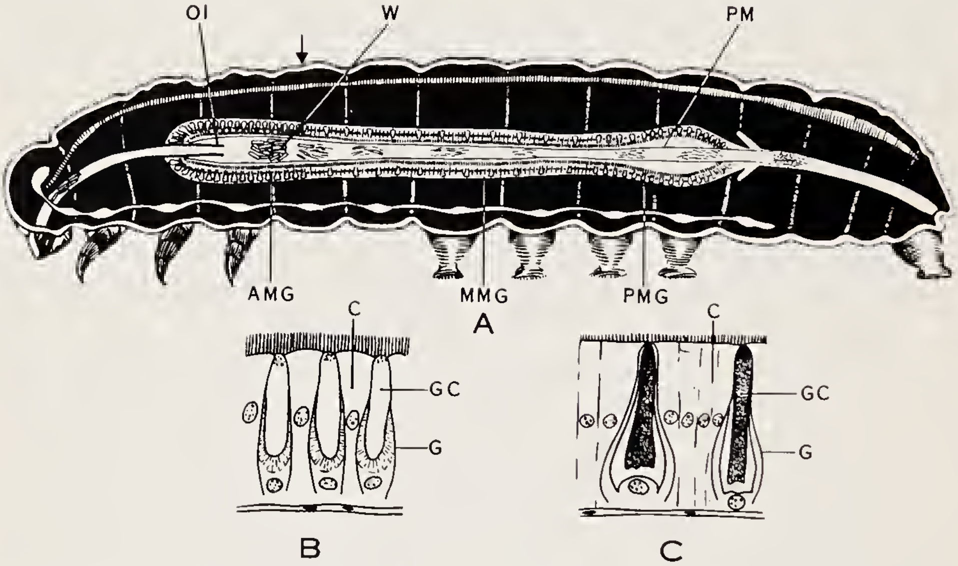


Fig. 2. (A) Diagram of optical section through a *Tineola* larva: *OI*, oesophageal invagination; *W*, wool fragments; *PM*, peritrophic membrance; *AMG*, anterior midgut; *MMG*, mid midgut; *PMG*, posterior midgut. (B) Epithelium of anterior or posterior midgut: C, columnar cell; GG, goblet cell cavity; G, goblet cell. (C) Epithelium of mid midgut. (B and C from Waterhouse (189)).

Wool fragments show no signs of digestion in the foregut. In *Tineola* they are generally assembled into bundles before being passed through the esophageal invagination into the midgut in which they are enclosed within the thin-walled peritropic membrane, which has a typical fibrillar network structure (96). The histology of the middle half of the midgut differs from the anterior and posterior quarters in having a columnar epithelium interspersed with few flask-shaped goblet cells, compared with a fairly regular alternation of columnar and cigar-shaped goblet cells (Fig. 2). When observed under polarized light, the wool fragments in the anterior quarter of the midgut are seen to be apparently unchanged and highly birefringent. At about the beginning of the middle region of the midgut (indicated by the arrow in Fig. 2) the birefringence (and, hence, the degree of orientation of the fiber components) diminishes abruptly, and much of it has been lost by the time the food has reached the end of the midgut. Where the abrupt change occurs, scales may be seen separating from the fibers, and striations between the cortical cells become conspicuous, presumably due to the removal of some of the cementing matrix. Isolated cortical cells may also be observed. The soft orthocortex is attacked far more readily than the paracortex (129). The fate of the scale cells is of interest, for they are more resistant than cortical cells to *in vitro* digestion (84) and to attack by microorganisms (122). In the posterior midgut, isolated scales are not visible after staining (36,151), and in the hindgut fragments of wool fiber are often not found, digestion having been complete (36). This is the typical picture. However, in larvae feeding rapidly some fragments are only partially digested and may be seen in the voided faecal pellets. In larvae which have been starved for a few hours, fresh food may reach the posterior end of the midgut in 2 hours (189), and fragments pass completely through the alimentary canal in 8 hours at 27°C. (36). The comparable figure for dermestid larvae is 8–12 hours at 30°C. (192).

Much the same changes occur in wool undergoing digestion in the dermestid gut. However, the conspicuous and abrupt change in birefringence at a particular level in the midgut does not occur, probably because the wool fragments are not "packeted" and thus not maintained in order of ingestion (192).

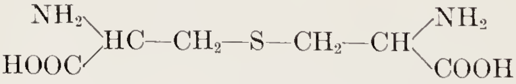
Since some of the disulfide linkages are converted to thiol groups both in fabric and in the tip end of fleece, Day (36) examined *Tineola* larvae feeding on the basal region of freshly shorn fleece in which all disulfide linkages were formerly thought to be intact, although this is now questioned. These fibers were attacked in just the same way as those from fabrics.

B. EFFECT OF MECHANICAL DAMAGE ON DIGESTIBILITY OF WOOL

In the intact fiber there are, as we have seen, not only serious mechanical barriers to the penetration of digestive juices but also various chemical linkages in the keratin molecule which are relatively resistant to enzyme attack. When wool is powdered by prolonged grinding in a ball mill, it is changed both physically and chemically, some of the cystine being converted to sulfate (160,162). This powdered keratin is partly digestible and will support rats if supplemented with tryptophane, methionine, histidine, and lysine (161). The cut ends of wool fibers are always the first to show signs of attack by enzymes (151). Nevertheless, the biting of wool into small fragments and other mechanical insults to which it is normally exposed do not explain the ability of wool-digesting insects to degrade keratin. Thus, if wool is contaminated with beeswax, it is eaten by the larvae of the waxmoth (*Galleria*) (108); if cut into 100 μ lengths and soaked with cabbage juice, it is ingested by cabbage white butterfly (*Pieris rapae* (L.)) larvae; and if dipped in sugar solution, the fragments are readily swallowed by the cockroach *Periplaneta americana* (L.). In each instance the fragments are excreted in a substantially unchanged condition, although there is some separation of cortical cells at the cut ends of the fibers, particularly in the case of *Galleria* larvae.

C. EFFECT OF ALKALINITY ON DIGESTIBILITY OF WOOL

Wool is susceptible to degradation under alkaline conditions with the eventual production of smaller peptides and of amino acids, including lanthionine,

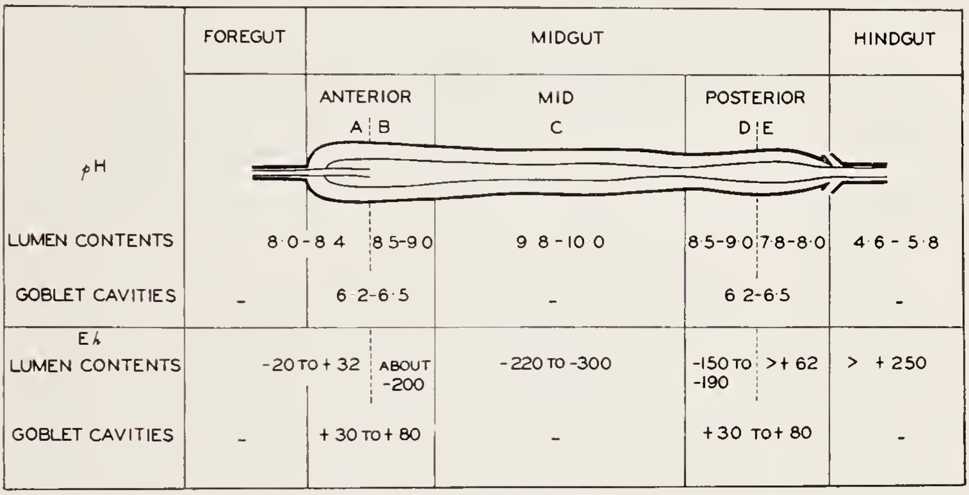


$$\begin{matrix}NH_2 \\ HOOC\end{matrix}\!\!>HC{-}CH_2{-}S{-}CH_2{-}CH\!<\!\!\begin{matrix}NH_2 \\ COOH\end{matrix}$$

formed by the elimination of one S atom from a cystine residue (4,31). Most of these changes occur at elevated temperatures or at more alkaline pH's than 11. In spite of the fact that the thioether linkages of lanthionine should be more resistant to reductive degradation than the disulfide bonds they replace, alkali-treated wool is more easily digested by papain in the presence of bisulfite than is normal wool (15). This indicates that changes have occurred in the molecule in addition to those involving the disulfide bond.

It has been known for many years that the foregut and midgut contents of *Tineola* larvae are alkaline, whereas the hindgut contents are acid (131,164,166,177). Duspiva (38) found that pooled midgut secretions ranged in pH between 9.44 and 10.15, with an average of

9.9, and Waterhouse (191) showed that the pH varied in different portions of the midgut, with a range of 9.8–10.0 in the most alkaline region, the mid midgut (Fig. 3). How important are these alkaline conditions in the process of wool digestion? It is known that midgut alkalinity is characteristic of all lepidopterous larvae and occurs also in some other insects (188). The pH of the midgut digestive juices of *Galleria* and *Pieris* larvae is 8.4 (38) and 9.4–9.7(197), respectively; and, as we have seen, no appreciable attack on wool occurred, although, admittedly, the pH is slightly lower than in *Tineola*. However, the pH of the midgut contents of the wool-digesting Dermestids *A. piceus An. verbasci*, *An. australis* (192), and *An. vorax* (149) falls in the pH range 6.8–7.0 and that of some bird Mallophaga is about 8.0 (194). There is good evidence, then, that, although alkaline digestive juices facilitate wool digestion, they can play no crucial part in this process.



	FOREGUT	MIDGUT					HINDGUT
		ANTERIOR		MID	POSTERIOR		
pH		A	B	C	D	E	
LUMEN CONTENTS		8·0-8·4	8·5-9·0	9·8-10·0	8·5-9·0	7·8-8·0	4·6-5·8
GOBLET CAVITIES	–	6·2-6·5		–	6·2-6·5		–
Eh LUMEN CONTENTS		-20 TO +32	ABOUT -200	-220 TO -300	-150 TO -190	>+62	> +250
GOBLET CAVITIES	–	+30 TO +80		–	+30 TO +80		–

Fig. 3. Diagram showing pH and oxidation-reduction potential (E_h in mv.) of various regions of the digestive tract of *Tineola* larvae (from Waterhouse (191)).

D. CHARACTERISTICS OF THE PROTEOLYTIC ENZYMES PRESENT

Mechanically sound, chemically unchanged wool fibers are highly resistant to the action of enzymes under sterile conditions. Pepsin and papain have no effect whatever; and, although trypsin, chymotrypsin, and pancreatin disintegrate the fibers very slowly, little protein (less than 10% is digested (4,63).

The proteolytic enzymes of *Tineola* have been examined to learn whether they possess any characteristics which might merit the designation keratinase (39,108,146). Linderstrøm-Lang and Duspiva (39,108) obtained from *Tineola* larvae an extract of considerable proteolytic activity, the midgut epithelium being found to contain about double the enzyme concentration of the digestive juices. The *Tineola* proteinase differed from vertebrate pancreatic trypsin in not being inhibited by thiol compounds, a property shared to a lesser degree by the proteinase from *Galleria* larvae. This thiol insensitivity was considered to be a useful adaptation to the substrate because of the high concentration of cystine in the diet (108), although more recent work indicates that crystalline trypsin is insensitive to the presence of cysteine (184).

Powning *et al.* (147) used eleven criteria by which to compare proteinase preparations from *Tineola* and four other insects (*Periplaneta, Locusta, Tenebrio,* and *Musca*) with trypsin and papain. From a study of the effects of a number of proteinase activators and inhibitors—namely, cyanide, thioglycollic acid, iodoacetate, ovomucoid, enterokinase, *Ascaris* inhibitor, and soybean inhibitor—it was concluded that, in general, the insect enzymes are similar and resemble trypsin more closely than papain. This conclusion was further strengthened by an examination of their pH optima, heat stability, and milk-clotting ability. There were some interesting, but noncritical, differences, such as the pH optimum of 9.8–10.0 for the *Tineola* enzyme, compared with 8.5 for trypsin and the other insect proteinases. The insect enzymes were all found to function well over the entire range of physiological oxidation-reduction potentials. This survey, then, gave no reason to believe that the explanation of the special ability of *Tineola* larvae to digest keratin resided in the peculiarity of its protein-digesting enzymes. However, Powning and Irzykiewicz (148) have now shown that a partially purified proteinase, used at a concentration similar to that in the *Tineola* midgut, does have activity which could justify the name keratinase. Under anaerobic conditions, but in the absence of reducing agents, the *Tineola* enzyme solubilized about 30% of the wool substrate in two days at 37°C. Under the same conditions crystalline trypsin had no effect. Both enzyme preparations had similar activity against casein.

Duspiva (39) also examined the enzyme extract from *Tineola* for aminopolypeptidase and dipeptidase. Both were present; and, as might be expected for enzymes involved in the splitting of small, soluble breakdown products, their concentrations were considerably higher (20 ×) in the midgut epithelium than in the digestive juices.

No information is available for wool-digesting dermestids except that a proteinase and a lipase occur in the midgut of *An vorax* (149) and that the proteinase concentration is similar to that in the *Tineola* midgut (148).

E. THE ROLE OF REDUCING CONDITIONS

The disulfide bond of wool cystine may be reduced by a variety of reagents (e.g., thioglycollate, bisulfite, sulfide), although the reaction is not necessarily confined to this bond alone. With thioglycollate two thiol groups are formed, one cystine residue being split into two of cysteine:

$$\text{Wool—S—S—wool} \rightarrow \text{wool—SH} + \text{HS—wool}$$

This reaction proceeds more rapidly under alkaline than under neutral or acid conditions. The redox potential (E'_0) of the cystine-cysteine system at pH 10 was first reported to be in the vicinity of -350 mv. (58), although a more recent determination indicates that it is closer to -400 mv. (175a). An important point to stress is that reduced wool is readily degraded by many proteolytic enzymes which have little or no effect on untreated wool. It is not safe to generalize, however, for the action of pancreatin is far less influenced by the rupture of disulfide linkages than by changes in the configuration of the wool molecules produced in other ways (42). If the reduced wool is exposed to appropriate oxidizing conditions, the disulfide bonds are reformed.

It was first pointed out by Linderstrøm-Lang and Duspiva (107, 108) for *Tineola* and since confirmed by Waterhouse (191,192,194) for many keratin-digesting insects that unusually reducing conditions are maintained in the larval midgut (*Tineola:* -220 to -280 mv. at pH 9.8–10.0; dermestids: -190 to -230 mv. at pH 7.0; bird Mallophaga: -200 mv. at pH 8.0). There is some variation in E_h in different midgut regions in *Tineola* (see Fig. 3) and Mallophaga, but not in dermestid larvae. Whereas the keratin-digesting bird Mallophaga maintain reducing conditions in the midgut, closely related mammal-infesting species which cannot digest keratin maintain oxidizing conditions (194). A similar difference exists between dermestid larvae which can and those which cannot digest keratin (192,197). So far as is known the midgut juices of other insects are oxidizing. The ability to maintain reducing conditions in the midgut digestive juices is the only unusual feature known to be common to keratin-digesting insects and is sufficient to explain their special ability to utilize keratin as a food.

Some attention has been paid to the factors responsible for the initiation and maintenance of the low oxidation-reduction potential, but their identity is still unknown. An intense nitroprusside reaction is only obtained in *Tineola* and wool-digesting dermestids if the test is carried out immediately after dissection (108,192). Reduced oxidation-reduction indicators in the midgut lumen can similarly be seen to change rapidly to their oxidized colors after a larva is opened (197). If a larval midgut is kept in a moist chamber, wool in the lumen undergoes little further change (108). Similarly, no digestion of wool was observed even when placed in a *Tineola* proteinase preparation at pH 10–11, although casein was readily degraded under these conditions. However, the addition of thioglycollate leads to partial digestion of the wool and heat inactivation of the enzyme prevents this. It is clear, therefore, that dissection of the larvae has abolished the reducing conditions and, with them, substantially all of the digestion of keratin. A little digestion is evidently still possible, as shown by recent tests involving wool exposed to purified *Tineola* proteinase preparations (148).

Larvae without food in the midgut or those feeding on casein (which is poor in sulfur) gave a very weak nitroprusside reaction, although indicators fed with the casein showed that the reducing conditions were still maintained (108). It was concluded that thiol groups were not involved in the system for poising the midgut redox potential.

Care is needed in the interpretation of tests with tetrazolium salts to indicate reducing conditions. If fed, these salts are reduced to insoluble formazans in the gut lumen under even mildly reducing conditions. However, if larvae are dissected under tetrazolium solutions the formazan may be produced in the epithelium by dehydrogenase systems with the formation of intensely colored deposits, as in the gut of the blowfly larvae (195,198) in the lumen of which the redox potential is always oxidizing. Wool-digesting insects reduce tetrazolium salts fed with their diet (191,192). Colored formazan is readily formed in the anterior midgut epithelium of *Tineola* (36) but does not form in the absence of added substrate in dermestid larvae (192).

It should be pointed out that the presence of —SH groups in the *Tineola* midgut does not necessarily mean that a reduction process is responsible, for it is known that small amounts of hydrolysis can occur with wool even in water (163). However, the rapid rate at which digestion occurs in the *Tineola* gut and the presence of adequately reducing conditions leave no reasonable doubt that the latter are responsible for the appearance of thiol groups. The occurrence of a low redox potential in the midgut does not necessarily result in the reduction of all materials introduced into the lumen or imply that, if reduction occurs, this will be a simple reaction. Such considerations led Linderstrøm-Lang and Duspiva (108) to suggest that there might be present an enzyme system dehydrogenating an unknown substance and a carrier system transferring the hydrogen thus made available to the sulfur bridges of keratin. Cystine and glutathione reductases are known (124,138,158), and recent experiments indicate that disulfide reductases may be present in *Tineola* and *An. vorax* (148). Xanthine oxidase (dehydrogenase), which is very active in *Tineola* larvae, functions at, and assists in maintaining, a very low redox potential (67), but its distribution in the body and its weak activity in wool-digesting dermestid larvae suggest that it cannot play an important part in keratin breakdown (see p. 231) (89). There is now good reason to believe that the system responsible for maintaining the reducing conditions is a complex one.

All of the wool-digesting insects so far examined have poorly tracheated midguts (37,192). Tracheal supply may either be lacking over large areas of the epithelium (in dermestids and Mallophaga), or, as in *Tineola*, the supply may be poor compared with other caterpillars of similar size. The *A. piceus* midgut was better tracheated than that of other dermestid species examined. A poor tracheal supply might be expected to contribute to the ease of maintaining reducing conditions. Such conditions, however, are not the inevitable consequence of poor tracheation, since the midgut of mammal-infesting Mallophaga, whose digestive juices have a positive redox potential, is as poorly tracheated as those of bird Mallophaga, which maintain strongly reducing conditions (194). The digestive tract of larvae of the fly *Sciara* is not tracheated (94), and it would be interesting to know the redox potential of its digestive juices.

F. EXCRETION

A knowledge of the excretory products of wool-digesting larvae throws some interesting light on the processes of digestion. Powning (144) has carried out careful analyses, considerably extending the work of earlier authors (15,85,135,149,164,177). Some of his figures are shown in Table VI.

TABLE VI
Analyses of Excreta of *Tineola* and *A. piceus* Fed on Woolen Fabric
(After Powning (144))

	Tineola	Attagenus
	(per cent dry weight)	
Total nitrogen	24.9	22.5
Soluble nitrogen	21.1	17.8
Amino nitrogen	1.2	—
Uric acid	41.0	2.8
Ammonia	4.1	0.97
Urea	3.0	3.0
Total sulfur	4.5	
Soluble sulfur	3.1	
Soluble SO_4 sulfur	0.24	
Insoluble sulfur	1.4	
Total cystine	10.0	
Soluble cystine	6.2	11.8

The three common nitrogenous excretory products uric acid, urea, and ammonia are all present in *Tineola* and *A. piceus*. Uric acid, which is present to the extent of 41% in *Tineola* excreta, is a particularly economical means of excreting nitrogen from a dry diet. The low uric acid figure (2.8%) for *A. piceus* is notable because, although the water-soluble nitrogen figures for both these insects are of the same order, ammonia, urea, and uric acid can account for about 86% of soluble nitrogen from *Tineola*, but only about 17% from *A. piceus*. Allantoin, which is excreted instead of uric acid by blowfly larvae (22,156), was not detected in *A. piceus*, and neither creatine nor creatinine were found in *Tineola* excreta (144).

In view of the very high uric acid content in *Tineola* excreta one would expect the enzyme xanthine oxidase to be present. This enzyme catalyzes the production of uric acid from xanthine or hypoxanthine and has a very wide distribution in the animal kingdom (48). An interesting feature is that the hypoxanthine–uric acid reaction has one of the lowest redox potentials recorded in a biological system (67). An extremely active xanthine oxidase with a pH optimum of 7.7–8.0 was found in *Tineola* larvae and appeared to be fairly generally distributed throughout the body, but with no particular concentration in the midgut. Because of the latter fact and of the finding that *An. australis* and *An. vorax* larvae have a rather weak xanthine oxidase (correlated with their lower contents of uric acid, 8.7% and 1.1% in faeces, respectively), there is little evidence that xanthine oxidase is intimately involved in the maintenance of reducing conditions in the gut lumen.

Although excretion of nitrogen in the form of urea is less economical in terms of water than is uric acid and although urea generally occurs in small quantities only in insect faeces, nevertheless 3% was found in both *Tineola* and *Attagenus* excreta. Analyses showed, furthermore, that the fresh *Tineola* midgut contained 0.14% urea (144). Urea is known to have a denaturing effect on proteins if the concentration is high enough (3–5M) (86,175), and, in the presence of reducing agents, it also increases the digestion of wool by some enzymes (105). If all the urea found in the *Tineola* midgut were concentrated in the short region where wool breakdown first becomes noticeable, the local concentration would be about 0.5M (144). At this concentration the rate of digestion of wool by urea-activated papain increases by one-third (105). Although wool digestion certainly cannot be explained by the presence of urea, there is good reason to believe that the amount present contributes to the general conditions in the gut lumen which are favorable for this process.

The fate of sulfur, in which keratin is unusually rich, is of considerable interest. About 55% of the total water-soluble sulfur excreted by *Tineola* is cystine sulfur, and only 8% is sulfate sulfur (Table VI). The remainder is unaccounted for. This contrasts with an earlier claim (almost certainly incorrect) for *T. pellionella* that cystine was absent and that the sulfur of keratin was excreted mainly as inorganic sulfate (164). It is clear from the table that cystine is a far more abundant excretory product than sulfate in *Tineola* (6.2%) and *Attagenus* (11.8%), as it is also in *A. vorax* (149).

Of the 1.4% insoluble sulfur, 1.0% is due to cystine in the undigested wool residues. It is probable that the remaining 0.4% is in the form of elemental sulfur (148). It is possible that this sulfur is formed by the action of hydrogen sulfide (liberated by cysteine desulfhydrase (144) or, possibly, by hydrolysis of wood cystine (163)) on the disulfide bonds of cystine, thus

$$R—S—S—R + H_2S \rightarrow 2\ RSH + S$$

The presence of free sulfur in the faeces is yet another unusual feature of wool digestion by *Tineola.* Since dermestid larvae do not possess an active cysteine desulfhydrase (144), it is not anticipated that free sulfur will occur in appreciable amounts in their faeces unless some other mechanism is involved.

The presence in the faeces of cystine rather than cysteine is not at all surprising, for cysteine in solution is oxidized to cystine by oxygen and this evidently occurs soon after a larva is dissected, as demonstrated by the rapid disappearance of the nitroprusside reaction. Moreover, the hindgut contents of *Tineola* (191) and wool-digesting dermestids (192) have a high redox potential so that this oxidation normally occurs in the hindgut before the food residues are excreted. The fact that water is released to the larva by this reaction (81,83) is a useful but purely fortuitous consequence of the passage of the cysteine out of the very reducing conditions necessary for its continued existence.

The water-insoluble fraction of *Tineola* excreta (mainly undigested wool fragments) contained 12.5% cystine, compared with 10.7% for the woolen fabric on which the larvae fed (144). There is little doubt that this is due to the differential digestion of wool fractions of different chemical composition, structures of highest cystine content being the most resistant to breakdown.

Lanthionine is produced from cystine when wool is exposed to the action of alkali (see p. 224), but it was not detected in *Tineola* excreta (144).

One might, perhaps, expect that any ammonia present would be associated with the excretion of sulfate. However, only about 0.1% ammonia would be required to combine with the small quantity of sulfate in *Tineola* excreta (144). Babcock (15) suggested that sulfur dioxide might be a metabolic product of *T. pellionella* larvae feeding on wool, but no trace of this material was found in *Tineola* (144).

G. DETOXICATION AND STORAGE EXCRETION

Valuable information on conditions in the gut during wool digestion have been obtained from a study of certain detoxifying mechanisms.

It was pointed out earlier that the thiol groups of reduced wool can be rebuilt into disulfide bonds by oxidizing agent. When rebuilding is carried out in the presence of appropriate metal salts, wool—S—metal—S—wool linkages are thought to be formed (43, 174). If the salt of a metal such as mercury is used, the resulting modified wool might be expected either to be indigestible or, if digested with consequent rupture of metal-sulfur linkages, to be highly toxic. Waterhouse (189,193) investigated the fate of thirty metallic and five nonmetallic elements, some built into the wool structure and others added by dipping the fabric into an appropriate solution. Nineteen elements that form insoluble sulfides produced characteristically colored sulfides in the food undergoing digestion in the *Tineola* midgut, and much of the sulfide formed passed down the digestive tract and was excreted. The absence of sulfide formation by other elements could be explained, e.g., aluminium, cerium, chromium, and zirconium do not form sulfides in the presence of water. The formation of sulfide by zinc but not by manganese was surprising, although the pH of the gut may be too high for the formation of manganese sulfide which is, in any case, far more soluble than zinc sulfide. Many metals expected to poison the larvae did not do so, and even arsenic and thallium were not as toxic as might have been anticipated. There is good evidence that considerable detoxification occurred by formation of insoluble sulfides.

When the *Tineola* larva is fed on nickel-impregnated fabric, there is much less cystine in the excreta (1.4%) than when it is fed on untreated cloth (6.7%). This suggests that the sulfides are formed from H_2S produced from cysteine in the midgut. Since cysteine is available in vast excess over the requirements of the larva, detoxification clearly occurs without drawing on materials which the larva would otherwise utilize. *Tineola* larvae have, in fact, a highly active desulfhydrase (pH optimum, 8.9) concentrated in the gut which liberates H_2S (and probably pyruvic acid and ammonia) from L-cysteine (145). When inhibitors of cysteine desulfhydrase (hydroxylamine or semicarbazide) are fed, distinctly less sulfide is produced than usual (192). It is interesting to note that pyruvic acid is present in both

Tineola and *A. piceus* excreta (144). *Tineola* desulfhydrase appears to be highly specific, since cystine, methionine, homocystine, and glutathione do not function as substrates. A similar, although far less active, enzyme is present in some other insects (e.g., *An. vorax*).

Although much of the sulfide formed is discharged with the food residues, a clearly visible amount accumulates in the form of granules in the cavities of the goblet cells (189). As shown in Figure 2, the goblet cavity is closed to the gut lumen by a fine membrane which does not carry the striated border typical of the adjacent columnar cells. The nature of the contents of the cavity is unknown, although it is certain that mucoid material is not present (35), and mucoid material is typical of the similarly named cells in the mammalian digestive tract to which the insect cells bear a slight superficial resemblance. Ascorbic acid is also absent from the goblet cells (33). The goblet cells are generally separated from one another by one or more columnar cells. In *Tineola* (Fig. 2) the goblet cells are much more numerous in the anterior and posterior quarters than in the middle region of the midgut (36,114,189,190).

Tellurium and metals such as zinc, nickel, lead, antimony, osmium, mercury, platinum, and gold formed sulfides which were strongly accumulated in the goblet cell cavities. Most of these accumulated only in the anterior and posterior midgut, although tellurium, mercury, and gold led to accumulations also in the tips of the flask-shaped goblet cells of the middle midgut. Functional differences in accumulations (especially of iron, nickel, and mercury) were also observed between zones of goblet cells in the anterior and posterior midgut, although these cells all had a very similar morphological appearance (189).

Available evidence indicates that the goblet cell cavities are closed. Either the sulfides must be formed there (and this is highly improbable because some of the ions, e.g., mercury and lead, are highly toxic) or these characteristically colored compounds must be absorbed in solution. At first sight the uptake of "insoluble" sulfides in this fashion may appear improbable, but this is what is believed to occur. It is thought that the formation of sulfides, in the presence of amino acids and polypeptides liberated by digestion of the food or present in the digestive secretions, leads to the formation of highly dispersed colloidal solutions in the same fashion as has been described *in vitro* by Neuberg and Mandl (136). Supporting evidence comes from the fact that a bluish green fluid typical in color of the ferrous sulfide–cysteine dispersion can sometimes be observed in the lumen after larvae have fed on iron-enriched fabric. Iron and copper are somewhat unusual in that they are accumulated in the goblet cells far more strongly than the color of the cell, indicating the presence of sulfide, would suggest. Either the color of the sulfides is masked or these metals occur in some other form. A very high proportion of the lumen surface is made up by the columnar cells, and it is to be expected, therefore, that most of the products of digestion will be absorbed by these cells. There is some evidence to suggest that the dispersed sulfides are taken up by the columnar cells and passed laterally to the goblet cells which discharge them into their cavities (189). Redox and pH indicators are also accumulated in the goblet cell cavities in the anterior and posterior midgut and an E_h of -20 to $+32$ mv. at a pH of 6.2–6.5 is indicated (Fig. 3). If, therefore, the normal contents of the cavities play any part in the maintenance of the E_h and pH in the digestive juices, it must be in the production of precursors of the systems responsible rather than in the production of the actual systems operative. However, in view of the fact that the goblet cells occur less frequently in the middle region of the midgut where the potential is lowest and the pH highest, it appears improbable that they play any important role in the maintenance of these conditions (191). So far as is known, goblet cells occur only in larvae of Lepidoptera. In this order, as in some others, there is no continuous regeneration of midgut cells; instead, either the entire epithelium is cast off and completely regenerated at each molt or it is at least extensively reorganized (114). If periodic discharges of goblet cell accumulations occur between moults in *Tinoela,* they cannot, on the basis of observations with sulfides, occur more than once every two or three days. Although accumulations of soluble dyes gradually disappear after larvae are transferred to undyed fabric, the rate is slow (191). There is thus little evidence to support earlier suggestions that the goblet cells are secretory and function principally in the production of digestive enzymes which are stored in the goblet cavity prior to discharge into the gut lumen (36,114,200). On the other hand, there is very good evidence that an important function is that of storage excretion, since goblet cells accumulate metals also in other caterpillars living on their normal diets (190).

There are some metals, such as barium and beryllium, that are toxic to many animals but form readily hydrolyzable sulfides. Some evidence exists that these may be detoxified by deposition in granules (mainly as insoluble phosphates) principally in the columnar cells of the anterior midgut. Granules of calcium and magnesium phosphates commonly occur in this region. Beryllium in amounts in excess of those capable of deposition as phosphate still has little toxicity for *Tineola* larvae. It is highly toxic to mammals and inhibits alkaline phosphatase and other magnesium-activated enzymes. Little alkaline phosphatase occurs in the midgut epithelium of *Tineola* (34), and this may possibly be correlated with the low toxicity of beryllium.

The midgut of wool-digesting dermestid larvae is simple, without differentiation into zones, and the functional epithelium consists of simple columnar cells. When larvae of *A. piceus, An. vorax, An. verbasci,* and *An. australis* eat woolen fabrics impregnated with many of the salts used for *Tineola* (e.g., those of Ni, Fe, Cd, Cu, Pb, Hg), the faeces produced are normal in color. There are two exceptions, namely, cobalt, when the faeces are colored by a dark brown water-soluble material, and tellurium, when a black insoluble material is present (192). It is clear that little or no H_2S is available in the gut for sulfide formation, and this appears to be correlated with the very low activity of cysteine desulfhydrase reported for *An. vorax* (144). In turn, the absence of desulfhydrase in dermestid larvae is clearly correlated with the far higher content of cystine in their excreta (11.8%) than in *Tineola* excreta (6.2%), for dermestid larvae evidently do not possess the capacity to split off very much of the unwanted excess sulfur. Evidence has been advanced in support of the hypothesis that, in dermestid larvae, many metals are complexed with cysteine (192). Most metal-cysteine complexes are not strongly colored, although cobalt and tellurium produce brown water-soluble and black precipitates, respectively. In view of the greater insolubility of most metal sulfides than their cysteine complexes, it is unlikely that the latter are formed in appreciable amounts in *Tineola* in spite of the fact that cysteine is freely available (189). It is interesting to note that bird-infesting Mallophaga also produce metal-cysteine complexes but not sulfides (194).

H. THE ROLE OF MICROORGANISMS

Larvae of *Tineola* normally harbor very small populations of microorganisms in their digestive tract. When fed on a diet containing casein, numbers of *Staphylococcus lactis* (which are often found in dairy products) were present in some cultures, and fungi were found in one culture on wool (29). Very few microorganisms were found in the digestive tract of *An. verbasci* (90). There is thus no reason to believe that microorganisms play a significant part in enabling keratin digestion by these insects. Titschack (180), in fact, claimed to have bred *Tineola* under sterile conditions on a diet of soiled woolen fabric.

It is, of course, well known that some fungi (e.g., the ringworm fungus *Microsporum gypseum*) and some bacteria are able to degrade keratin, although relatively little is known of the details of this process. Structural changes in wool undergoing attack by microorganisms are similar to those caused in the *Tineola* gut, except that the scales do not appear to be digested (122). Amino acids are liberated (32), and relatively large amounts of ammonia are produced (171). The cystine content of wool gradually increases during attack, indicating a differential removal of a protein fraction which is relatively poor in this amino acid. Soluble cystine is produced, although this is eventually converted into inorganic sulfate. No H_2S was detected (170).

An extremely active keratinase which does not cause the reductive cleavage of the disulfide linkage has recently been isolated from a species of *Streptomyces* (140). It is interesting to note also that protein disulfide reductase activity (on yeast cell wall preparations) has been demonstrated in certain yeasts (137) as well as active cystine and glutathione reductases (158). In general, however, proteinase preparations from keratinolytic microorganisms have not been found to have any effect on keratin (170,176). However, in these instances the role of reducing conditions in their action *in vivo* has not been investigated and there is almost no published information to indicate that they were looked for. No necessary correlation exists between resistance of various mothproofing treatments to microorganisms and to wool-digesting insects (209).

V. Digestion of Silk, Spongin, and Chitin

There are reports in the literature of the ability of dermestid larvae to digest silk, spongin, and chitin, although there is no satisfactory evidence to support these claims. Similarly, neither clothes moth nor dermestid larvae are able to digest cellulose fibers or artificial fibers of nonprotein origin.

Raw silk threads consist of two strands of water-insoluble silk fibroin (forming some 75% of the fiber) which are cemented together and surrounded by sericin as they are extruded from the paired larval silk glands. Sericin consists of one water-insoluble and two water-soluble fractions. Sulfur-containing amino acids have not been detected in silk, although there is a small amount of inorganic sulfide associated with the water-insoluble sericin (165). There is a high proportion (more than 80%) of low molecular weight amino acids in silk (glycine, alanine, serine, and tyrosine) (21), leading to a high degree of crystallinity of the protein. Silk is one of the "naked chain" fibrous proteins which carry little of their weight in side chains and are generally resistant to attack by digestive enzymes.

Abderhalden (1) reported that *Anthrenus museorum* larvae developed on silkworm cocoons, and this has led to the belief that the larvae are capable of digesting silk. However, there is reason to believe that they were living on contaminating materials rather than on silk itself (192).

An. australis larvae readily ate degummed silk (with water-soluble sericin fractions removed), but microscopical examination under ordinary and polarized light failed to reveal any indication that the fibers had been attacked by digestive enzymes, although there were many signs of mechanical damage caused during ingestion (192). Tyrosine, which is present to the extent of about 11% in fibroin, could not be detected in the faeces. If raw silk is ingested, the two water-soluble sericin fractions are likely to be removed during passage through the alimentary tract, and their amino acid composition (165) makes it not improbable that they may be attacked by digestive enzymes. However, just how much digestion may occur is not clear. Thus, Duspiva (40) found that, although sericinase-containing fluid regurgitated by the silkworm moth just before emergence from the cocoon dissolved the sericin and softened the silk, very little breakdown of sericin could be detected. On the other hand, Nisizawa and Ogawara (139) indicated that sericinase hydrolyzed the two water-soluble sericin fractions.

The position may be summarized by saying that, whereas dermestid larvae may be able to obtain some nourishment from the water-soluble constituents of raw silk and from silk contaminants, they do not appear to be able to digest processed silk (fibroin + water-insoluble sericin) which forms the great bulk of the raw silk fiber. Available evidence indicates that this statement also applies to *Tineola* larvae (192).

Spongin, which occurs in sponges, is a structural fibrous protein probably belonging to the collagen group, although it is sometimes classed as a pseudokeratin. It contains 1–2% iodine and, on hydrolysis, produces diiodotyrosine and dibromotyrosine (157). It is very resistant to digestive enzymes. There is, however, one report, which requires confirmation, that dermestid larvae are able to digest spongin (12). This is based on the reputed absence of fibrous particles in the faeces of *Dermestes frischii* and *D. lardarius* which had damaged sponges, although fibers were present in faeces from *Attagenus* species. However, there is no evidence that the *Dermestes* larvae actually ingested spongin in any quantity in spite of the damage caused to the sponges.

Chitin is a high polymer of *N*-acetylglucosamine units and is resistant to attack by most digestive enzymes, although it is degraded by some microorganisms. The well-known fact that various dermestid larvae (notably *An. verbasci*) are serious pests of insect collections has led to the suggestion that they may be able to digest chitin in which their diet is rich. However, no chitinase could be detected in extracts of the digestive tract of *An. verbasci;* both alimentary canal and excreta contained very few microorganisms and these were not chitinolytic; and no significant loss of chitin could be observed after larvae had fed on dead insects. It was concluded, therefore, that the larvae were unable to digest chitin (90).

VI. General Methods of Control

Passing mention only will be made of measures for controlling wool-digesting insects, except those which can be described under the heading of mothproofing. This term has come, by popular usage, to include proofing also against dermestid attack. In addition to good housekeeping procedures (regular sweeping, removal of debris, etc.) the following control methods may be listed; many references to their application to particular species are quoted by Hinton (80,83), and the most useful methods have been summarized (9,45). Where no comment is given the methods are not in routine use.

1. Physical means of control.
    a. High temperatures (above 40°C.) (150) are lethal, although susceptibility varies with the species.
    b. Cold storage of furs is an effective way of preventing damage. Feeding ceases at low temperatures, but many species can withstand relatively long periods of exposure to cold.
    c. Vacuum.
    d. X-rays.
2. Chemicals for control.
    a. Lures and traps.
    b. Fumigants. Hydrogen cyanide, methyl bromide, and a number of other materials are effective fumigants and are used commercially for disinfestation. The vapors of *p*-dichlorobenzene and naphthalene are also toxic (13,14), although adequate concentrations are seldom attained by the householder.
    c. Repellents. Storage of woolens in cedar wood chests (102) and camphor, *p*-dichlorobenzene, and naphthalene have been used to repel attacks by wool-digesting insects. Except for clean fabrics stored in tightly fitting chests, these methods are not very effective.
    d. Sprays and dusts. DDT, lindane, chlordane, and dieldrin are all extremely valuable insecticides and very considerable protection can be obtained if they are properly used (9). Either effective mothproofing, or the use of such toxicants combined with good housekeeping procedures, is the most desirable and efficient means of control of damage by wool-digesting insects.
3. Biological control. There is no information to indicate that parasites or predators either exercise or could be made to exercise more than occasional control over wool-eating insects. A number of hymenopterous and a few dipterous parasites of wool-digesting insects have been recorded, in addition to several predaceous mites (64,69,80,83,127,207). *Nosema* and gregarines attack various species (64,114,115), and a polyhedral virus attacks *Tineola* larvae (116,167,168).

VII. Methods for Assessing Mothproofing Agents

Almost all mothproofing methods involve the use of stomach or contact poisons. In order to assess the relative efficiency of different proofing materials it has been necessary to develop reliable methods for biological testing of the treated fabrics. These methods are now used quite extensively not only for the evaluation of new materials but also for the routine checking of the so-called "permanent" mothproofing processes by commercial firms. The gradual development of testing methods has been discussed by many authors (7, 47,73,74,76,173,185–187,211).

It is probably true that dermestid larvae are generally more resistant to toxicants than moth larvae. Certainly this applies to pentachlorophenol and Mitin (127). However, it is impossible to generalize with DDT (see p. 256); Eulan CN is low concentrations is more effective against *An. vorax* than *Tineola* (17); and certain commercial quaternary ammonium compounds are needed in twice the concentration for protection against *Tineola* as against *A. piceus* (44,127).

Because mothproofing agents may vary greatly in their effectiveness of protection against attack by different species, the trend now is to use *Tineola bisselliella*, *A. piceus*, and *An. vorax* in tests. It is probable that *Hofmannophila* will be added to the list, for large larvae of the species have recently been shown to be almost unaffected by one widely used agent (Lanoc CN) which is effective against other species. Testing methods differ in detail, but the following, tentatively adopted by the International Wool Textile Organisation (10), combines most of the desirable features.

Ten pieces of the sample to be tested and five each of a standard fabric impregnated with 0.4 and 1.2% 2,4-dinitro-α-naphthol, respectively (as reference mothproofing treatments), are submitted in separate containers to larval attack. Three other samples of standard untreated cloth, exposed to identical conditions but without larvae, make it possible to correct for alterations in weight of the test objects occasioned by humidity changes. With *T. bisselliella* 15 larvae of total weight 13–17 mg. are placed on each sample for 14 days at 23–25° C., controlled to $\pm 0.5°$C., and 65–70% R.H., controlled to $\pm 3\%$ R.H. The surviving larvae are then counted and the silk webbing and faeces removed from the samples which are weighed to determine the amount of fabric lost. It is suggested that a fabric should be considered as possessing a reasonable level of mothproofness if no more damage is caused than to a fabric containing 0.8% 2,4-dinitro-α-naphthol. No comparable suggestions have yet been made for carpet beetle attack. However, in the official Swiss method (6), 20 *An. vorax* larvae of average weight 0.8 ± 0.1 mg. are used per sample, the temperature is maintained at 28 ± 1°C., and the "permissible" damage is 15% of untreated controls.

Numbers and size of larvae, temperature, humidity, duration of test, and methods of assessing protection have been subject to much variation. Visual methods of assessment are no longer regarded as reliable and, in addition, do not provide quantitative data. Frass weight has been found a useful index of attack in the case of dermestid larvae but not for moth larvae where uncontaminated frass is more difficult to separate. Mosher (135) suggested that an estimation of the uric acid produced would be an accurate measure of the amount of food eaten, but a careful re-examination of this method demonstrated that the results obtained were more variable than those from the weight-loss method (147). To reduce variability in tests the insects must be bred under suitable, carefully standardized conditions. Tests using highly inbred stocks of *T. bisselliella* surprisingly indicated no advantage over normal laboratory cultures (147).

VIII. Mothproofing Methods

The ideal mothproofing agent must satisfy a number of conditions, of which the following are probably the most important. It must (*1*) be highly toxic to wool-digesting insects, but nontoxic or only very slightly toxic to mammals at the concentrations employed; (*2*) be resistant to repeated washing and drycleaning; (*3*) be resistant to the hazards of use, e.g., light, rubbing, pressing, perspiration, sea water, etc.; (*4*) be odorless; (*5*) produce no undesirable changes in the properties of wool; (*6*) have no deleterious effect on dyes or dyeing properties of wool; (*7*) be capable of being introduced into the dye bath during normal dyeing operations; and (*8*) be cheap.

So many and varied have been the compounds claimed to have mothproofing properties that it is only possible to deal with the most interesting or effective methods. Further details on other agents will be found in Clark (27), Moncrieff (132), and Roark *et al.* (152–155).

The first logical attempt to replace the many ineffective household remedies which had been used for centuries was commenced 50 years ago by Meckbach (125) of the Bayer Company (27,132). From an investigation of an old saying among German housewives that green-colored materials were not attacked (although improved green dyes introduced a few years earlier did not confer protection) Meckbach discovered that the obsolete dyestuff Martius yellow (2,4-dinitro-α-naphthol, Fig. 4), the yellow component of the old green dye, had mothproofing properties. His first aim of producing a range of dyes which would also confer mothproofing qualities was later replaced by that of finding a colorless chemical which could be used in conjunction with any dye.

A. FLUORIDES

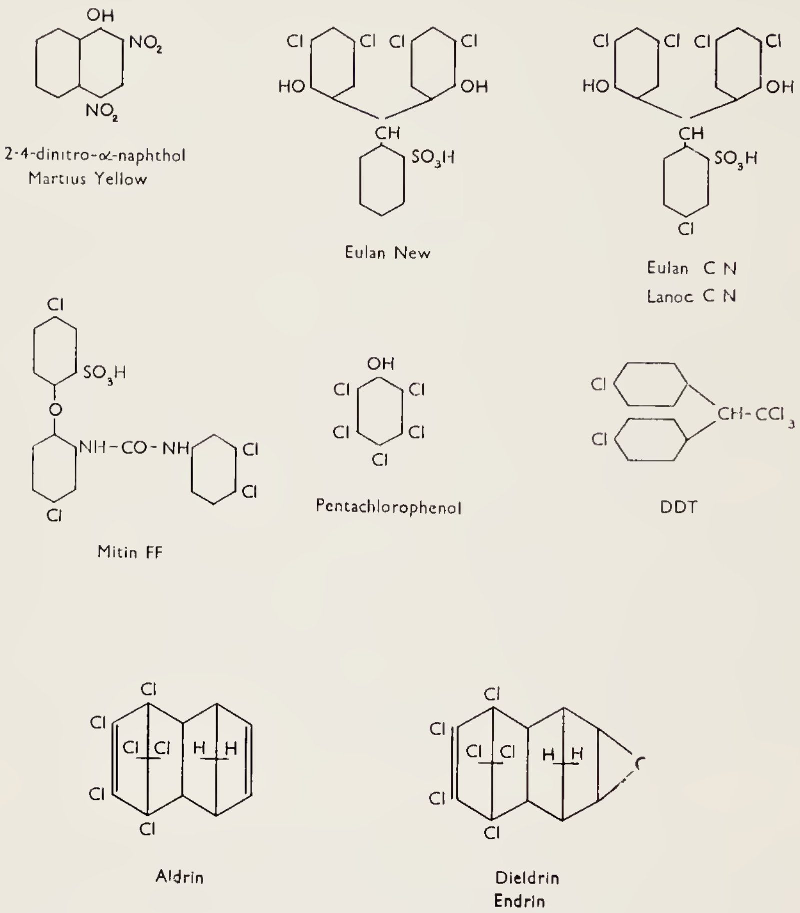
The first colorless materials of promise were inorganic fluorides and included many compounds still regarded as highly toxic to moth larvae. Since 1920 there has been a large number of patents claiming to have overcome the various difficulties associated with the use of fluorides, such as the fact that they readily powder or brush off and are easily removed by washing. The first fluorine-containing mothproofing agent, Eulan M, was marketed in 1920 and was followed in relatively quick succession by Eulans F, Extra, W (a double fluoride of aluminum and ammonia), and W extra (potassium bifluoride). The last-named remained on the market until 1939 by virtue of its low cost and high efficiency where fastness to water or the presence of acidity or inorganic salts did not matter. Many further fluorides were patented by other groups: chromium fluoride (claimed to confer better resistance to washing), a mixture of chromium and antimony fluorides (for circumstances where the chromium salt alone could not be used with pastel shades), sodium antimony fluoride, triethanolamine silicofluoride (because of lower toxicity of silicofluorides to humans), sodium silicofluoride (which causes some harshening of wool), potassium borofluoroacetate, and 1,6-diamino-2,2-difluorohexane (Boconize) (27,73,132).

Although many of these compounds are adequately toxic to wool-digesting insects providing 0.2–0.3% fluorine is present (73), they are not in widespread use today because of their potential toxicity to humans (the inorganic compounds) and their lack of persistence under many conditions of normal use.

B. TRIPHENYL METHANES

The first of the colorless mothproofing triphenyl methanes (Eulan New, Fig. 4) appeared in 1928 (27,73,132). Other members of this series were dyestuffs (most being nontoxic) and, like them, Eulan New was applied from an acid dyebath from which it exhausts almost completely, the sulfonic group conferring not only water solubility but also substantivity to the wool fiber. It was reasonably fast to all agencies except severe milling, provided a good measure of mothproofing at 3% by weight of the fabric, and strengthened the fibers against abrasion.

This was replaced in 1934 by Eulan CN (the same as Lanoc CN of ICI) which differed from Eulan New by possessing an extra chlorine atom in the para position of the sulfonated benzene ring (Fig. 4). Eulan CN has the advantages of being a better mothproofing agent (although still used at 2–3% by weight), being faster to washing, and drawing effectively from a more weakly acid dye bath, so that it can generally be applied without additional processes during normal dyeing operations. The current cost of mothproofing with Lanoc CN is about 5 cents per pound of wool. It is claimed that the proof is fast to washing, drycleaning, light, rubbing, hot pressing, bleaching, perspiration, carbonizing, and sea water. Both Eulan New and Eulan CN also confer on wool a certain amount of resistance to bacterial degradation. Eulan CN (Lanoc CN) suffers from two disadvantages as an all-purpose mothproofing agent



2-4-dinitro-α-naphthol
Martius Yellow

Eulan New

Eulan CN
Lanoc CN

Mitin FF

Pentachlorophenol

DDT

Aldrin

Dieldrin
Endrin

Fig. 4. Structural formulas of a number of mothproofing agents.

(8). One is that, although initially colorless, it discolors slowly under the influence of light; however, there is apparently no loss in biological efficiency. Stability to light has recently been claimed following the introduction of methoxy groups into the nucleus. The other disadvantage is that it does not confer protection against any but the youngest *Hofmannophila* larvae.

C. MITIN FF

Mitin FF, another water-soluble colorless dyestuff (Fig. 4), was introduced by the Geigy Company in 1939 (23,46,104,169, 211,212). It confers much the same type of protection and possesses the other desirable properties of Eulan CN, although costing slightly more. Three per cent by weight of the wool gives satisfactory protection against moth larvae, whereas 4% is required for proof against dermestid larvae (66). This is equivalent to 1% of the purer Mitin FF (highly concentrated) preparation which is in current commercial use. Notable features are that Mitin FF can be applied from a neutral as well as an acid dye bath, that it does not discolor on exposure to light, and that it confers some protection against attack by *Hofmannophila.* These desirable properties offset its slightly higher cost. Protection is not impaired by soiling of the fabric with organic matter.

D. PENTACHLOROPHENOL

This material (Fig. 4) has been used for some time for the protection of wool against fungal and insect attack. More recently it has been used for mothproofing in emulsion form (Mystox B of Catomance Ltd.) or as a pentachlorophenol laurate–protein complex (Mystox LS), which is claimed to be more resistant to washing and to leave no smell. Ordinarily 0.5–0.8% is used to confer moth resistance, although 1.6% is required to protect against dermestid attack. Even at 1.6% it is cheaper than Lanoc CN or Mitin FF (73,132). However, its resistance to washing is poor, and it tends to become brown on exposure to light (65,132); furthermore, it is comparatively volatile, so some is lost if the textile is subjected to heat. Pentachlorophenol preparations find their main application for deeper shades of furnishings, etc., which are unlikely to be washed and, for such uses, confer excellent protection (132,169).

E. DDT

A valuable review, to the end of 1952, of work on the factors involved in the use of DDT (Fig. 4) for mothproofing has been published by Wälchli (186). Only very small amounts (of the order of 0.03%) need be present on wool fibers to confer considerable resistance to attack (186), although, for practical purposes, 0.1–0.2% by weight is desirable (17,100,102). It is worth noting that 0.2% DDT effectively protects against damage by *Hofmannophila* (23).

Because of its insolubility in water, DDT must be applied as an emulsion or from a solvent. Despite earlier reports (65,91), DDT has some substantivity to wool when applied from certain dilute emulsions (100,132). One effective emulsion concentrate, known as EQ-53, contains 25% DDT, 10% nonionic emulsifier of the polyhydric alcohol or alcohol ether type (e.g., Triton X-100), and 65% aromatic hydrocarbon solvent (e.g., xylene) (100). If this is diluted greatly and used in the final rinse water from washing or dyeing, the DDT deposit is considerably greater than calculated from the amount of fluid absorbed by the cloth. Nonionic emulsifiers are absorbed by wool to a lesser extent than other detergents, and they may thus be more readily lost during rinsing to produce a more persistent DDT (199). If a deposit of 0.3–0.5% DDT is aimed at (which is still comparatively cheap), the fabrics remain proofed after several washings (65,199). DDT is considerably more resistant to washing than pentachlorphenol (65). DDT treatments are not resistant to drycleaning, but they are so cheap and easy to replace during the final solvent rinse that this raises no problem. In some countries many drycleaning establishments treat all woolens with DDT without extra charge. A DDT emulsion applied at the rate of 0.2–0.5% DDT to U. S. Quartermaster stocks of woolen fabrics has already given protection for 5 years and is saving more than 1.5 million dollars annually over previously used methods based on naphthalene, fumigation, and spraying (183). For this purpose DDT at 0.5% by weight is claimed to render better protection than any other commercial mothproofing compounds used at their recommended concentrations (99).

It is interesting to note the report that the wool of sheep dipped in a DDT preparation was resistant to moth attack even after several months' growth and weathering on the sheep's back (23).

F. DIELDRIN

Lipson and Hope (112,113) report that dieldrin (Fig. 4), applied in emulsion form at the rate of 0.05% by weight of the fabric, confers excellent mothproofing at very low cost. It is little affected by heat, although the concentration of dieldrin is considerably lowered by dyeing, bleaching, and milling. There is a slight removal during drycleaning, although sufficient remains for good protection. It is best used to treat goods which are usually drycleaned or only occasionally washed. It is not recommended for frequently washed goods such as underwear or baby wear. Wool treated with dieldrin has no odor, and the physical and chemical properties of the wool are unchanged. Endrin gives similar results, but aldrin (Fig. 4) is not as satisfactory. Endrin and dieldrin are isomeric and differ from aldrin in possessing an epoxy group.

Dieldrin does not react with wool but penetrates from the emulsion into the fiber when the latter is swollen by water. After the wool is dry, the dieldrin is not removed by the usual drycleaning solvents, none of which cause swelling of the fiber. However, it can be extracted completely with hot methanol or acetone, and it is gradually lost when wool is repeatedly washed in soap solutions (113a). There is a suggestion that sheep dips containing dieldrin may confer subsequent moth resistance on the wool (9,11, 93,112,113).

G. OTHER INSECTICIDES

Many other compounds have been patented or investigated as mothproofing agents (132), but none have found as widespread acceptance as those listed above. Among these one can mention Perthane and Strobane (101) chlordane, methoxychlor, toxaphene, benzene hexachloride (103), and formaldehyde (121). In most instances these are either inferior to, or at least no better than, the treatments outlined in more detail, and all depart in some important property from the requirements of an ideal agent.

H. MODIFICATION OF THE MOLECULAR STRUCTURE OF WOOL

A novel approach to the problem of mothproofing was introduced by Geiger and Harris (61). This was based on the knowledge that wool was very much more readily digested by proteolytic enzymes if the cystine cross linkages were reduced (63) resulting, as it were, in a depolymerization of the large three-dimensional molecules into smaller linear molecules. Depolymerization commonly leads to greater chemical reactivity (and, hence, ease of digestion), whereas condensation or polymerization is nearly always associated with greater stability and resistance to chemical attack. When the reduced wool was subsequently reoxidized, it regained its original stability. If an aliphatic dihalide was present, bis-thioether linkages (wool—S—$(CH_2)_n$—S—wool) were formed. Where appropriate halide compounds were used, the modification in wool structure resulted in greater chemical stability (toward alkalis, acids, and oxidizing and reducing agents) than untreated wool. Furthermore, these modified wools were much more resistant to biological assault in the form of clothes moth or carpet beetle larvae or of purified enzymes. Satisfactory alkylating agents include trimethylene dibromide and tetramethylene dibromide. Varying proportions of disulfide bonds could be converted into bis-thioether linkages by varying the amount of calcium thioglycollate used in the reducing bath. As shown in Table VII, there does not appear to be any advantage gained in modifying more than half of the cystine linkages. This may be because the steric configuration of the wool molecule is such that some of the cystine residues are readily vulnerable, whereas others are more or less protected and hence less reactive (132). Wool in which the cystine content had been reduced to 6.5% suffered only 2% with

TABLE VII

Susceptibility to Attack by *T. bisselliella* and *A. piceus* Larvae of Chemically Modified Wools Prepared by Reduction Followed by Alkylation with $(CH_2)_3Br_2$

(After Geiger *et al.* (62))

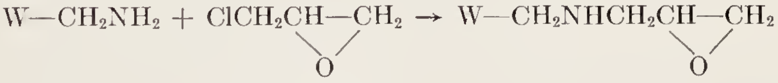
		Attack by larvae of	
	% Cystine	*Tineola* (Loss in fabric wt., %)	*A. piceus* (Faeces wt., mg.)
Untreated wool	12.2	15.5	52
Modified wool	10.0	8.0	45
	6.5	0.3	21
	4.0	2.9	34
	2.0	0.1	23

Tineola and 40% with *A. piceus* of the loss of weight of control wool with a cystine content of 12.2%.

Disadvantages of this method are the inadequate proofing against dermestid attack and the high cost of the treatment, which has not found widespread use. Some doubts on the mothproofing effectiveness have also been expressed (73,208,211). The disadvantage of high cost was removed when Harris and Brown (72) developed a very simple and cheap method for simultaneously rupturing sulfur linkages and rebuilding them, using a reducing agent such as sodium hydrosulfite and rebuilding agents such as ethylene dibromide, formaldehyde, and glyoxal. It is interesting to note that consecutive, as opposed to simultaneous, treatment with these compounds gives poor results.

More recently it has been shown (95) that the resistance of wool to the action of alkali can be improved by treatment with dihalogen compounds with exchangeable halogen atoms even without previous reduction of the cystine groups. The same result can also be secured by the action of di- or polyethylene derivatives on unreduced wool. The inference is that stabilizing linkages other than those involving a modification of the disulfide groups are produced in normal (and probably also in reduced) wool. Proof that other stabilizing linkages may be formed comes from various sources (4) including the following:

1. *Treatment with epichlorhydrin*, which is reported to confer resistance to moth attack (132) and which is postulated to proceed by reaction with free amino, phenolic hydroxyl, and carboxyl groups in wool (4), e.g.,



$$\underset{\text{Wool}}{\mathrm{W{-}CH_2NH_2}} + \underset{\text{Epichlorhydrin}}{\mathrm{ClCH_2CH{-}CH_2}\ (\text{-O-})} \rightarrow \underset{\text{Treated wool}}{\mathrm{W{-}CH_2NHCH_2CH{-}CH_2}\ (\text{-O-})}$$

Wool Epichlorhydrin Treated wool

More recent tests indicate that the protection against moth attack produced by this treatment is not of a high order (208).

2. *Treatment with glyoxal.* Since the diamino acids arginine, lysine, and histidine together comprise about 14% of the amino acids of wool, it was postulated that, if wool was treated with a suitable dialdehyde, the free amino groups would react to form Schiff bases, and new cross linkages would be formed as follows (132):

$$\mathrm{W{-}CH_2NH_2} + \underset{\text{Glyoxal}}{\mathrm{CHOCHO}} + \mathrm{NH_2CH_2{-}W} \rightarrow \underset{\text{Modified wool}}{\mathrm{W{-}CH_2N{=}CHCH{=}NCH_2{-}W}}$$

The new linkages would augment the natural disulfide bonds leading to a polymerization of the wool molecules, and this, in turn, would confer resistance to moth attack.

Glyoxal does, in fact, react readily with wool and was claimed to confer resistance to moth attack (132), although this requires confirmation. A surprising result was that about 0.1% glyoxal on the weight of the wool—about 1% of what might be expected to be needed on stoichiometric grounds—gave excellent results. Various explanations of this effect have been offered (132) of which the most attractive is that a very small quantity of glyoxal is theoretically capable of increasing the molecular weight of the wool considerably, providing the new bridges are mainly intermolecular; this increase in molecular weight (i.e., polymerization) then confers resistance to attack. The glyoxal treatment is claimed to be completely fast to washing and drycleaning, extremely cheap, and compatible with normal dyeing operations and not to affect the handle or appearance of wool. Various other aldehydes and diketones gave far less promising results (132).

None of these modifications of the molecular structure of wool have yet found any widespread acceptance as commercial mothproofing methods.

IX. Action of Mothproofing Agents

In view of the large amount of developmental work on mothproofing agents, surprisingly little is known about the mode of action of even the more outstanding materials. What information is available is summarized below. It is interesting to note that, to date, there have been no records of the development of insecticide resistance in any wool-digesting insect.

A. FLUORIDES

Wool dipped in 1–4% sodium fluoride is relatively distasteful to *Tineola* larvae and kills a high percentage after ingestion of only a small number of fragments, although some larvae survive for a time. Since calcium is often present in sufficient amount to permit the formation of calcium-rich granules in the midgut, it has been suggested that absorbed fluoride may be prevented from exerting toxic effects by rapid deposition as calcium fluoride in the granules. However, since the amount of calcium available is relatively small, the stage is soon reached when all ingested fluoride cannot be detoxified (189). Since *Tineola* larvae have an efficient mechanism for detoxifying toxic substances which form insoluble sulfides and to a lesser extent those which form insoluble phosphates, fluorine compounds would appear to offer better possibilities for mothproofing than any other inorganic materials. It is significant in this regard that fluorides are the only inorganic materials which have been widely used in recent years for mothproofing fabrics.

B. EULAN CN (LANOC CN)

Reumuth (151) reports that Eulan CN–treated fabric is repellent to *Tineola* larvae which can be induced only with great difficulty (and following gross soiling) to ingest pieces of wool. In particular instances where one, two, and four small (0.1 mm.) pieces of treated wool were ingested no signs of digestion were observed. Other sources claim that this agent is a stomach poison (8,23) and that some wool is eaten, the amount consumed depending upon the concentration of the toxicant. This is more in line with Reumuth's own findings that larvae which take one or two bites of Eulan-treated wool show a choking with crystals (possibly uric acid) of the alimentary canal and malphigian tubules and a discharge of a viscous sticky excrement which makes their movements very slow and may trap the larvae. This is probably the explanation of the circular spinnings having a radius that of the length of the larva which are typical of larvae on Eulan CN–treated materal. Production of silk is evidently not greatly impaired.

Larvae placed on treated fabric tend to molt or pupate sooner than expected and are reported to starve to death rather than eat any appreciable amount of Eulan-impregnated wool. The avoidance of treated material is easily demonstrated by exposing both treated and untreated fabric simultaneously to larval attack, which leads to the destruction of the latter and no recognizable signs of damage to the former. When a mixed fabric of treated wool and some other fiber is exposed, it is said that both fibers are protected against damage (118), although this probably depends upon the manner in which the fibers are blended.

C. MITIN FF

Valuable information on the mechanism of the protection conferred by Mitin against *Tineola* larvae has been obtained by Lotmar (117). She obtained good evidence that the primary cause is the toxicity of treated fibers which are swallowed. However, since attack ceases while larvae still appear normal, a secondary repellent effect was postulated. Such has, in fact, been demonstrated by the observation that, when placed on a fabric consisting of proofed and unproofed wool, moth larvae will eat the untreated threads and leave the treated ones (211). Soiling of treated fabric does not influence its protective effect.

A specific toxic effect of Mitin is shown by the observations that starved larvae live longer than those on treated fabric and that larvae on fabric +10% Mitin die sooner than on fabric +3% Mitin (Table VIII) (117). If larvae are transferred to untreated wool after eating Mitin-treated wool, the majority die. On the other hand, when Mitin-treated and untreated fabrics are placed side by side and infested, slight damage may eventually be caused to the treated material by larvae which feed occasionally on it (23). The life of larvae may be prolonged at lower temperatures, but the protective effect of Mitin is the same.

TABLE VIII
Average Survival in Days at 23°C. of Starved *Tineola* Larvae and Those Fed on Mitin-Treated Fabric
(After Lotmar (117))

Larval size, mm.	Mitin on fabric		Starved
	3%	10%	
Small, 1.5–3.0	2.9		6.0
Medium, 4.0–6.0	4.3	2.6	11.0

Larvae immediately commenced eating when placed on wool treated with 3% Mitin which, if suitably dyed, could be seen shortly after in the alimentary canal. However, some hours later feeding ceased (earlier on 10% Mitin than on 3%), and the gut progressively emptied over the next day or two. Larvae feeding on untreated wool fairly regularly produced, during their life cycle, 10–15 faecal pellets per day (except when molting) with an average total of 700 per larva. Starved larvae excreted an average of 3.7 pellets during the emptying of the content of the alimentary canal. Comparable larvae placed on dye fabric containing either 3 or 10% Mitin produced average totals of 5.3 and 2.3 pellets, respectively, equivalent to $1^1/_2$ and $^2/_3$ gutfuls of treated food which must have been ingested. Compared with the 700 faecal pellets produced by normal larvae these figures show that the amount of treated wool swallowed is less than 1% of that consumed by control larvae, which amounts in practice to negligible damage. It must also be noted that the younger the larva, the more susceptible it is to Mitin, so that the deposition of moth eggs on a treated fabric would lead to still less damage (117). In some instances there may be a starvation effect superimposed upon the toxicity of any Mitin which has been ingested, for occasionally larvae may live for 20 days on treated fabric (206). In order to investigate the influence of Mitin on digestive processes, larvae were starved until the midgut no longer gave a positive nitroprusside reaction for —SH groups and then fed on Mitin-treated wool. After 7 hours, keratin digestion appeared to be progressing normally and a positive nitroprusside test was given. After 24 hours some larvae no longer gave a positive test, the proportion which did not react increasing progressively with time. Often there were larvae with undigested and partially digested fibers in their midgut which gave no nitroprusside reaction (117). This last observation probably means that the midgut redox potential had risen to a level at which the —SH groups of the partially digested wool were oxidized and hence rendered unreactive to nitroprusside.

No histopathological changes were observed in the blood, alimentary canal, or other tissues of larvae poisoned with Mitin. Larvae which have eaten Mitin-treated fabric tend to molt earlier than usual, although death generally ensues before the old cuticle is shed. The few larvae which manage to molt successfully will not attack Mitin-treated wool subsequently.

As with moth larvae, newly emerged dermestid larvae soon die on Mitin-treated cloth, whereas older larvae are very much more resistant both to poisoning and to starvation (23,46,66). The majority of three-month-old and older *A. pellio* larvae survived 28 days forced exposure on Mitin-treated wool and a very small amount of excrement from the material was voided (Table IX). Furthermore, when transferred to untreated fabric, many were able to develop normally.

Mitin, therefore, tends to repel these larvae when they have ingested a sublethal dose, and, under natural conditions, there is little doubt that they would migrate elsewhere (23). According to Luttringhaus (118) both Eulan CN and Mitin protect to about the same degree against *Attagenus* larvae (the amount of excrement is approximately the same), although the mortality caused by the Eulan is twice that caused by Mitin.

TABLE IX
Experiments with *A. pellio* at 72°F., 55% R.H. for 28 Days
(After Burgess (23))

		3.5% Mitin			Untreated fabric			Starved	
Larval weight, mg.	Larval length, mm.	Faeces wt., mg.	Loss in fabric wt., mg.	Mortality, %	Faeces wt., mg.	Loss in fabric wt., mg.	Mortality, %	Faeces wt., mg.	Mortality, %
0.91	3.5	0.7	3.9	62	6.4	18.6	0	0.6	0
1.61	4.2	0.9	6.4	32	11.6	26.4	0	0.9	0
3.40	5.2	1.3	9.0	24	20.3	44.6	0	1.0	0
5.84	6.4	3.2	11.6	14	25.0	51.9	0	2.9	0

Tests with larvae of *A. piceus*, *An. vorax*, *An. verbasci*, and *An. australis* gave similar results, the larvae producing very little excrement, although survival was high. In one test with *An. australis* 119 larvae out of 140 survived 28 days exposure on Mitin-treated wool which had been subjected to aging, laundering, and drycleaning and produced negligible damage during this time (23).

Mitin can be regarded as specifically toxic to keratin-digesting insects when it is attached to the wool fiber. Thus, insects which cannot digest wool, such as larvae of the yellow mealworm *Tenebrio molitor* L., can eat Mitin-treated wool without being harmed, fragments of undigested wool being present in the faeces. Furthermore, *Tenebrio* larvae damaged samples treated with 4, 5, and 6% Mitin just as much as untreated control samples (211).

D. DDT

DDT acts both as a contact and as a stomach poison against clothes moth larvae. There is no reason to believe that its ultimate mode of action is any different from that in other insects, for there are many common features. These include typical tremors and slow death; greater susceptibility of younger (or freshly molted) than older larvae; and less damage to DDT-treated fabrics at lower than at higher temperatures, owing to a less vigorous attack by the larvae and a higher toxicity of DDT at the lower temperatures.

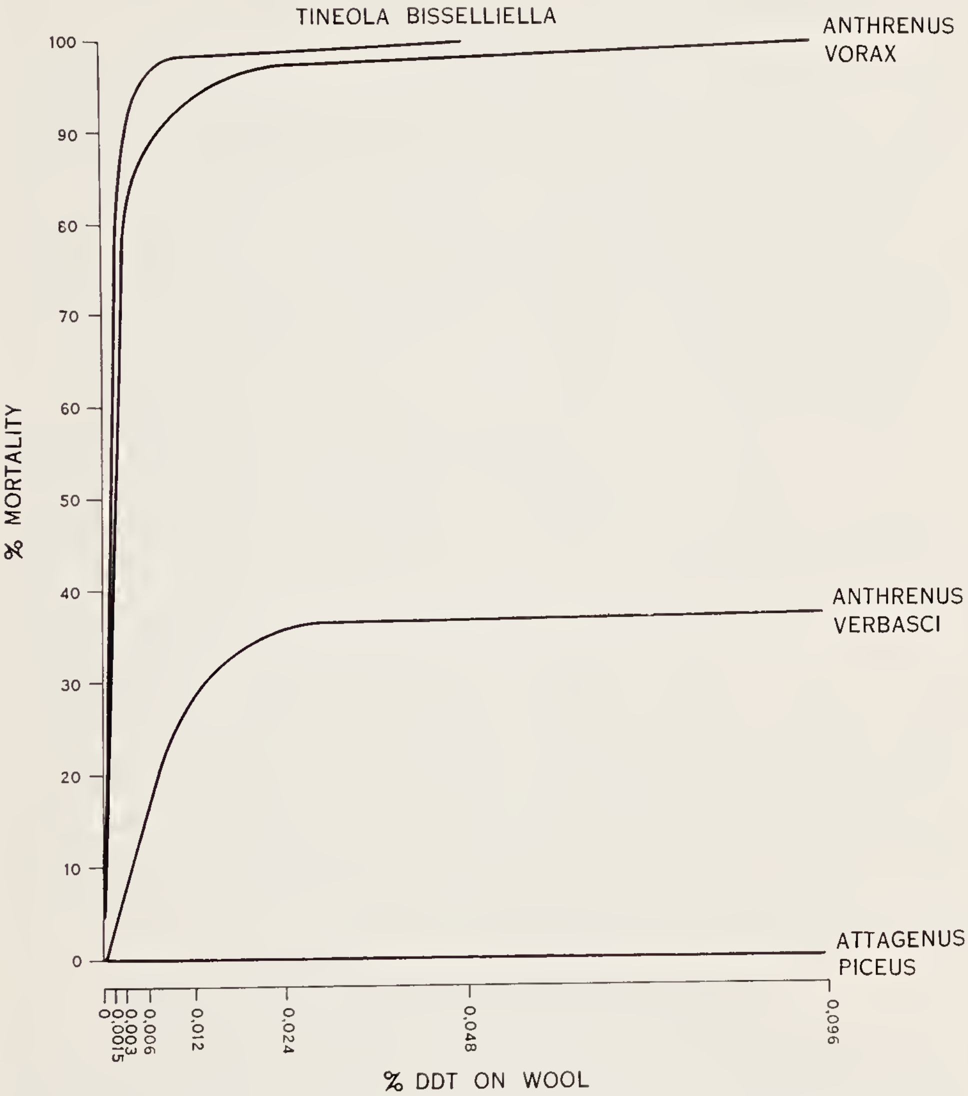


Fig. 5. Mortality of various wool-digesting insects as a function of DDT concentration on woolen fabric (after Wälchli (186)).

DDT has no repellent effect on clothes moth adults, which can mate and lay eggs on treated fabric (e.g., containing 0.2% DDT by weight) before being killed (23,66). These eggs hatch normally, but the young larvae do not survive for long. In one experiment 18 larvae emerging from eggs placed on fabric impregnated with 0.2% DDT had an average life of 37 hours. Fourteen moved off the fabric, all but one showing abnormal contortions, and 11 died in a more or less vertical position with their posterior ends stuck to the glass dish by an anal excretion. No wool was detected in the alimentary canal and no silk was spun. Some large larvae of *T. bisselliella* and *T. pellionella* will survive contact with DDT if they are not given the opportunity to ingest treated fabrics (23). However, it is clear that larvae are affected either by contact with, or after ingestion of negligible amounts of, fabric containing adequate amounts of DDT (0.075% or more) (23).

Half-grown larvae of *Anthrenocerus australis, An. verbasci,* and *A. pellio* survive for several weeks on wool containing 0.08% DDT, but they are adversely affected and deterred from eating the wool (23,66). It has been suggested that the far greater hairiness of dermestid larvae compared with moth larvae helps to protect them against as intimate contact with the DDT-treated surface (186). However, as shown in Figure 5, this is by no means the whole story, for there are considerable differences in susceptibility among dermestid species. Relative to attack on untreated cloth, *An. verbasci* ate treated fabric most heavily, followed by *Tineola* and *A. piceus,* with *A. vorax* causing very little damage. A higher concentration of DDT is required to protect a fabric against damage by *An. verbasci* than against the other species, but some individuals are killed; *A. piceus* does a little damage, but without suffering any mortality even on fabrics containing 0.01% DDT (186).

E. MODIFICATION OF MOLECULAR STRUCTURE

Day (36) examined the digestive tracts of *Tineola* larvae feeding on woolen fabric in which bis-thioether linkages had been substituted for some of the disulfide linkages. The only visible signs of digestion of fibers in the gut were striations in the cortex. The scales were intact even at the posterior end of the midgut. There is good reason to believe, therefore, that the new linkages introduced into the wool molecule had largely prevented the alkaline reducing conditions in the midgut fluid from exposing partly degraded wool to attack by proteolytic enzymes.

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RELATION OF CHEMICAL STRUCTURE TO ACTIVITY FOR 2,4-D-TYPE HERBICIDE AND PLANT GROWTH REGULATOR

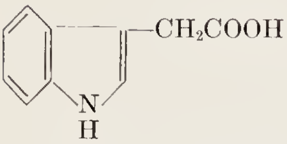
By R. L. WAIN, *Wye, Kent, England*

CONTENTS

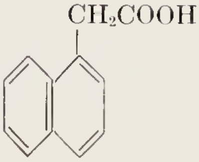
I. Introduction 263
II. Methods of Assessing Growth-Regulating Activity 266
III. Chemical Structure in Relation to Growth-Regulating Activity 269
A. The Carboxyl (—COOH) Group 270
B. The Unsaturated Ring System 273
C. The Side Chain 273
D. Unsaturated Side Chains 279
E. Substitution into the Ring System of Phenoxy Acids 280
F. Optical Isomers and Growth-Regulating Activity 287
G. Influence on Activity of Side Chain Length 290
H. Activity in Homologous Series 291
I. Amide and Nitrile Derivatives of ω-(Aryloxy)alkanecarboxylic Acids 298
IV. Conclusion 301
References 301

I. Introduction

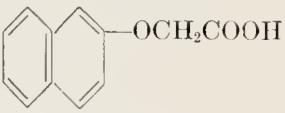
The spectacular developments which have taken place with hormone weed killers over the past twenty years stem from the discovery by Kögl and his coworkers (65) in 1934 that indole-3-acetic acid is present in human urine and that this substance is markedly active in promoting cell elongation in plants. These important findings were followed in the same year by the isolation of indoleacetic acid from yeast by Kögl and Kostermans (66), and in 1935 Thimann (112) showed that the acid is also produced by the fungus *Rhizopus suinus*. It was some years, however, before the compound was found in higher plants (7,43,44). In the meantime, the simple nature of the indoleacetic acid molecule and its high activity as a growth substance had led rapidly to the examination of other compounds of related structure. In 1935 further indole compounds were reported to be active by Hitchcock (50) and Kögl and Kostermans (67), and in the same year Zimmerman and Wilcoxon (148) found 1-naphthylacetic acid and a number of other arylacetic acids to possess growth-regulating properties.



Indole-3-acetic acid

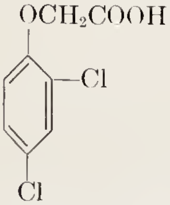


1-Naphthylacetic acid

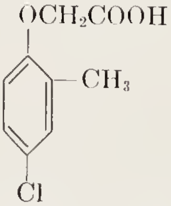


2-Naphthoxyacetic acid

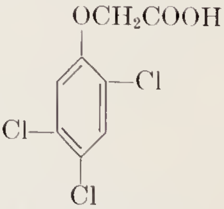
Then came the demonstration by Irvine (54) that 2-naphthoxyacetic acid is a growth substance, and this stimulated interest in the phenoxy acids. The extremely high activity shown by some of these compounds, such as 2,4-dichlorophenoxyacetic acid (2,4-D), was first reported by Zimmerman and Hitchcock (145) in 1942 who, at the same time, showed that growth-promoting activity can be exhibited by certain derivatives of benzoic acid (145). It is among the phenoxy acids that most of the hormone-type selective weed killers, such as 2,4-D, 2-methyl-4-chlorophenoxyacetic acid (MCPA), and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), have been found.



2,4-Dichlorophenoxyacetic acid
(2,4-D)



2-Methyl-4-chlorophenoxyacetic acid
(MCPA)



2,4,5-Trichlorophenoxyacetic acid
(2,4,5-T)

These synthetic materials, however, are best referred to as plant growth substances (or auxins), and only those substances which actually occur within the normal plant, e.g., the natural auxins indole-3-acetic acid, and indole-3-acetonitrile (isolated from cabbage by Jones *et al.* (55) in 1952) should rightly be considered as plant growth hormones.

At the present time a large number of active plant growth substances are known, and much attention has been given to studies on the relationships which may exist between chemical structure and plant growth-regulating activity. These investigations have been concerned almost entirely with aryl and aryloxy acids and their derivatives, and a number of reviews of this work have been published (6,56,63,84,114,119,123,124,128). Although a number of simpler aliphatic compounds, such as carbon monoxide, ethylene, acetylene, and maleic hydrazide, are capable of producing growth responses in plants, little is yet known of the mode of action of these substances, and they will not be considered further in the present account.

If an active molecule is to produce a growth response when applied to a plant or segment of living plant tissue, it is reasonable to assume that (*1*) the chemical must be capable of entering the tissues; (*2*) once inside, it should be able to move toward and accumulate at the site of action within the cells, and it should not be inactivated by chemical reaction or held at "unproductive" sites en route; and (*3*) having reached the site of action at cell level, its molecule should possess the structural characteristics which are necessary for the initiation of a growth response (this activity at site of action has been termed "primary activity" (119)). Many physical and chemical processes are involved in the sequence of events which determine the mode of action of a growth substance, and the chemical structure of the active molecule is all important. For this reason, it is possible for the organic chemist to modify growth-promoting activity by the introduction of specific groupings into the molecule and by other means. In spite of much research, however, only little is known about the precise mode of action of substances which induce growth responses in plants. Some workers consider that the mechanism is essentially physicochemical; thus, for example, Veldstra (120) has suggested that growth regulation arises from an association between the active molecule and the lipoid portion of the colloidal protoplasmic boundary of the cell whereby permeability and flow of essential metabolites are increased (see also 17,18,124). Other investigators favor the view, first put forward by Skoog *et al.* (104), that the primary function of a growth substance is that of a coenzyme in some essential metabolic process, such as nucleic acid metabolism (38,39, 102,103), or processes in which phosphorylation (15,36) or the activity of sulfhydryl groups is involved (74,75,114). Another suggestion recently put forward is that growth-regulating substances produce the physiological response by acting as chelators of metallic ions within the cell (48,49). In particular, it is considered that the calcium-pectin relationship in the cell wall might be affected by such a chelating mechanism (10). These views have received some support (138), but the suggestion that all growth substances can act in this way has been questioned (31). Whatever the mechanism underlying the growth response, however, it is known that the mode of action is complex and that any basic understanding of the physiological and biochemical processes involved must await the results of long-term research on all aspects of this problem. A study of the chemical structure of molecules in relation to their growth-regulating activity as determined by standard tests represents only one such aspect. All workers in this field, of course, appreciate that the importance of molecular structure is not solely related to the "primary" activity of the molecule at site of action, for, as already indicated, physical properties are also involved. Furthermore, it is recognized that a compound having entered plant tissues might be enzymically converted to a substance showing a different order of activity from that which it would itself have exhibited. Such factors cannot easily be taken into account when interpreting experimental results, but in spite of these and other fundamental limitations, research on structure-activity relationships has led to interesting speculations on mode of action from which have arisen new growth substances, some of which are finding important uses in agriculture.

II. Methods of Assessing Growth-Regulating Activity

A variety of test methods are available for detecting and assessing plant growth responses induced by treatment with these materials. Some are laboratory tests carried out on excised plant material, for example, the *Avena* or wheat straight growth tests (12,14,30,107,116) based on linear growth measurements of cylindrical pieces of oat or wheat coleoptile immersed in a solution of the growth substance (Fig. 1), and the pea curvature test (30,60,93,107,140,142), which depends upon measurement of inward curvature induced in divided pea shoots placed in a solution of the substance (Fig. 2). It has been suggested (123) that, in such immersion tests, the ability of the chemical to reach the site of action within the tissues is not seriously complicated by such factors as penetration. Furthermore, these tests are well adapted for quantitative work and can give results which are highly reproducible. For these reasons, they have been widely used by workers in this field.

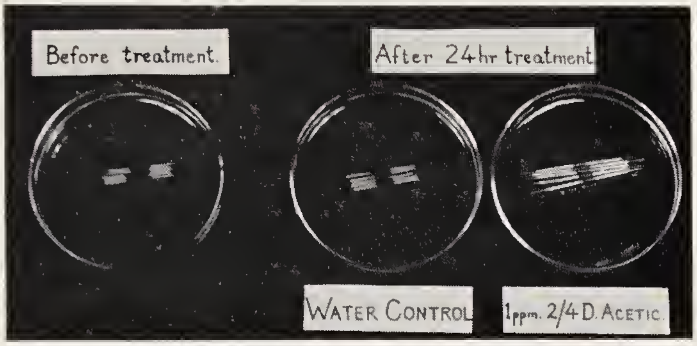

Before treatment.
After 24hr treatment.
WATER CONTROL
1ppm. 2/4 D. ACETIC.

Fig. 1. Growth response in the wheat straight growth test.

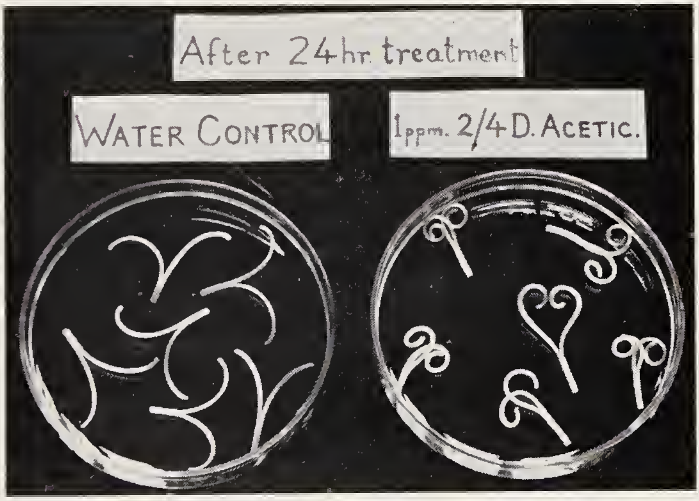

After 24hr treatment
WATER CONTROL
1ppm. 2/4 D. ACETIC.

Fig. 2. Pea curvature test. Segments of pea stem split for two thirds of their length bend outward in water but inward in a solution of a growth substance.

The first quantitative method for assessing growth-regulating activity was the *Avena* curvature test, designed by Went (139) in 1928. This involves the measurement of curvature induced in decapitated oat coleoptiles, when the substance in agar is placed on one side of the cut surface. For many years this was the standard procedure in most work on natural auxins. In general, however, it has been found that synthetic substances do not induce appreciable responses in this test (8). Another method of assessment is by the tomato leaf epinasty test (50,110,145) in which application of the growth substance in lanolin to the leaf axil, by promoting cell elongation, causes the angle between leaf petiole and main stem to increase (see Figs. 3 and 6). The tomato parthenocarpy test (92,106,147), depending on the production of seedless (parthenocarpic) fruit following application of growth substances to emasculated tomato flowers, provides another method of assessing growth-regulating activity.

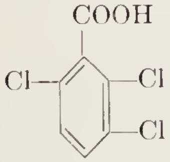
It has been long known that roots are very sensitive to auxins, but here, except at very low concentrations, the response is not one of growth promotion, as with coleoptiles, but inhibition. Some sensitive assay methods based on this inhibitory effect of auxins on root growth have been developed (1,4,5,81,144), but unless the biological material is rigidly standardized, variable results may be obtained (81,96). Other tests which have been used to assess growth-promoting activity include those based on the capacity of the compound to induce rooting responses in cuttings (51,76,81) or to produce morphogenic effects in plants (90,146).

It is often found that a compound does not show the same order of activity when examined in different tests. Thus, for example, in the *Avena* curvature test, indole-3-acetic acid is one thousand times more active than 2,4-dichlorophenoxyacetic acid (8), yet these acids show the same order of activity in the *Avena* straight growth test (133). Again, α-(1-naphthoxy)propionic acid is capable of inducing epinasty, morphogenic effects, and parthenocarpy in the tomato but produces negligible responses in the *Avena* straight growth and *Avena* curvature tests (87). Since, as already indicated, a primary requirement in growth-regulating activity is that the compound must possess the necessary physical properties and stability to reach the site of action, it is clear that a potentially active compound with low penetrative properties might appear inactive in certain tests (113,119,127). It must also be remembered that cell elongation, cell division, or both might be involved in the various types of growth response. Further, the rate of supply of water, sugars, and other essential substances might affect the magnitude of any such response. All these considerations, then, indicate that the status of a particular compound as a growth substance is more accurately assessed if a range of tests is used. High activity in a number of such tests justifies examination of the compound as a hormone-type herbicide (e.g., 86,111). Here intact growing plants are used; and, because of differences in morphological characteristics, enzyme makeup, and other factors, there may be differences in degree of response to the chemical in different species of plants. This provides the basis for the selective activity which is so important in this class of herbicide.

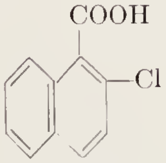
III. Chemical Structure in Relation to Growth-Regulating Activity

The first comprehensive studies in this field were made in 1938 by Koepfli, Thimann, and Went (63). They concluded from their investigations, using a range of compounds in the pea curvature test, that an active growth substance must possess a ring system containing at least one double bond with a side chain carrying a carboxyl (—COOH) group (or a grouping that may readily be converted to —COOH), there being at least one carbon atom between the ring and the —COOH group.

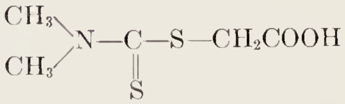
For many years these requirements proved useful as a guide to research in this field, but in the light of present knowledge they are not generally acceptable. Thus, inactive compounds are now known which on the above structural basis should be active; furthermore, there are compounds whose molecules do not fulfill these requirements which are active. For example, a number of benzoic acids (e.g., 2,3,6-trichlorobenzoic acid (11)), naphthoic acids (e.g., 2-chloro-1-naphthoic acid (122)), and certain acidic compounds not possessing a ring system (e.g., *S*-carboxymethyl dimethyldithiocarbamate (61)) are decidedly active as plant growth regulators, yet their molecules do not possess the structural features considered necessary by Koepfli *et al.*



2,3,6-Trichlorobenzoic acid



2-Chloro-1-naphthoic acid



S-Carboxymethyl dimethyldithiocarbamate

We must remember, however, that these structural requirements were put forward originally to explain activity in the indole- and arylalkanecarboxylic acids. In these groups of compounds, and in the corresponding aryloxy acids, if we ignore the influences on activity of secondary characteristics such as ring substitution and side chain length, the requirements still hold at the present time. The question arises, however, why these requirements are not satisfied by compounds such as the three whose formulas are given above.

It is often assumed that all organic acids which produce similar growth responses in plants share a common mode of action, and that any hypothesis which explains the activity of 2,4-D, for example, should also explain the activity of such diverse molecules as the above benzoic, naphthoic, and dithiocarbamate derivatives. It is by no means certain, however, that unrelated acids like these act in precisely the same way. Indeed, if we accept the possibility that a physiological response may arise from an activity of the molecule at one of a number of different points in a chain of reactions associated with growth, then the means by which this is achieved may differ with compounds of varying structure, though the ultimate effect on growth may be similar. Such considerations justify studies on the relationship between chemical structure and growth-regulating activity in one group of compounds, such as the aryloxyalkanecarboxylic acids.

We have seen that Koepfli *et al.* specified five minimum requirements for a compound to be active as a growth substance. These were modified by Veldstra (119,120), following investigations involving polarographic studies, to (*1*) a ring system with a high surface activity and (*2*) a carboxyl group in a very definite spatial position relative to the ring system (see also 56). Other suggestions have been made by workers in this field; but, before these are discussed, some consideration will be given to the various structural features which might be important in relation to plant growth-regulating activity.

A. THE CARBOXYL (—COOH) GROUP

Most of the growth substances which do not contain a —COOH group are derivatives of carboxylic acids such as esters, amides, and nitriles, all of which, it will be noted, might be converted to the parent acid within plant tissues. In many cases, these derivatives are less active in initiating a growth response than the acids themselves, though this is not always the case. Thus, in the *Avena* and wheat straight growth tests, indole-3-acetonitrile is more active than indole-3-acetic acid, whereas indole-3-acetamide is less active (13,100).

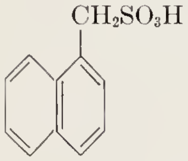
The superiority of the nitrile in this test may be due to a higher activity per se or to better penetration of the compound before it becomes hydrolyzed to the acid. On the other hand, 2,4-dichlorophenoxyacetic acid is more active than the corresponding nitrile in the straight growth test (28). This nitrile is also highly active in the pea curvature test, although indole-3-acetonitrile is inactive. Such findings are not consistent with the view that nitriles themselves produce the active response. Indeed, most of the evidence suggests that conversion to the parent acid is a prerequisite for activity with all these derivatives; and for this to occur, the specific enzymes necessary to effect the conversion must presumably be present in the tissues of the biological material used in the test. It has been shown by Seeley *et al.* (100), using chromatographic methods, that in pea tissues, in which indole-3-acetonitrile induces no growth response, conversion to indole-3-acetic acid does not occur. The corresponding amide, however, is readily hydrolyzed to the acid in this tissue, which is consistent with its activity in the pea curvature test. Furthermore, both the nitrile and amide are active in the wheat straight growth test, and each yields indole-3-acetic acid in this tissue (100).

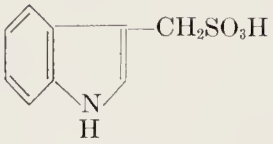
A range of 2,4-dichlorophenoxyacetyl derivatives of amino acids were investigated by Wood and Fontaine (143) who found that those derived from DL- and L- amino acids were active, whereas those from the corresponding D- acids showed only negligible activity. The same type of behavior was later found by Krewson *et al.* (69) with amino acid derivatives of α-(2,4-dichlorophenoxy)propionic acid. Assuming that a free carboxyl group is necessary for activity, then these results can be accounted for by an ability of the plant to hydrolyze the peptide link in L- but not D- derivatives.

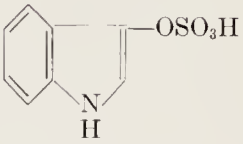
Other compounds which may show activity include those with terminal groups which can be converted to —COOH by oxidation, e.g., indole-3-ethanol (97) and indole-3-acetaldehyde (19).

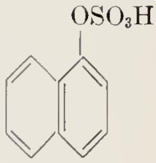
The effect on activity of replacing the —COOH group in a growth substance by other acidic groupings has been investigated by a number of workers.

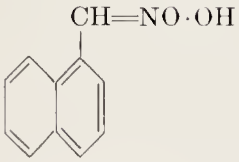
A number of sulfonic acids, e.g., 1-naphthylmethanesulfonic and indole-3-methanesulfonic acids, and the sulfuric esters of 3-hydroxyindole and 1-naphthol were found by Veldstra (123) to be slightly active at high concentrations in the pea test, as were the *aci-* form of 1-naphthylnitromethane (119) and a number of 1-naphthylphosphonic and -phosphonous acids (124). Weak growth-regulating activity was also found for 2-chlorophenoxymethylphosphinic acid by Maguire and Shaw (78; see also 41), while 1- and 2-naphthylmethylarsonic acids have been found in different tests to show an antiauxin effect (70).

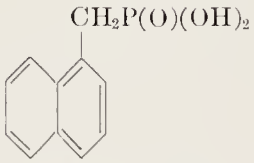

1-Naphthylmethanesulfonic acid

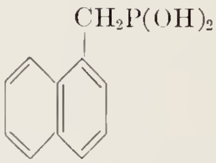

Indole-3-methanesulfonic acid

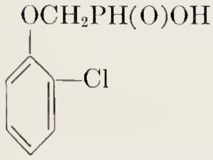

3-Indolylhydrogen sulfate

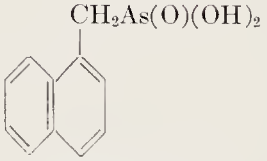

1-Naphthylhydrogen sulfate

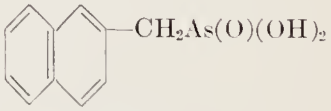

1-Naphthylnitromethane (*aci-* form)


1-Naphthylmethanephosphonic acid


1-Naphthylmethanephosphonous acid


2-Chlorophenoxymethylphosphinic acid

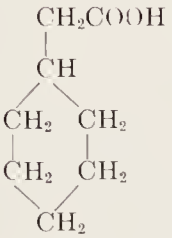

1-Naphthylmethanearsonic acid


2-Naphthylmethanearsonic acid

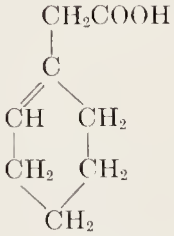
All these results indicate that while other negative polar groups can sometimes confer weak growth-regulating properties on compounds, their effect is greatly inferior to that of the —COOH group. It would therefore seem that for a compound to show high growth-regulating properties, its molecule should contain either a carboxyl group or a grouping which may readily be converted to carboxyl within the tissues of the plant. This, as already stated, was originally postulated by Koepfli *et al.* (63). It is of interest to note, however, that certain phenoxythiocarboxylic acids, such as those analogous to 2,4-D and 2,4,5-T, where the —COOH is replaced by —COSH, can show high activity (20).

B. THE UNSATURATED RING SYSTEM

One aspect of structure-activity relationships which appears to be of importance is the presence in the molecule of a ring system with at least one double bond (63) or a specific type of unsaturated aliphatic grouping (61). The molecules of active compounds such as indole-3-acetic acid, 1-naphthylacetic acid, and 2,4-D all contain an unsaturated ring system, whereas cyclohexylacetic acid, for example, in which the ring system is fully reduced, is completely devoid of activity. The presence of a single double bond in the ring can confer activity to the molecule, provided that its position is adjacent to the side chain (141).



Cyclohexylacetic acid
(Inactive)

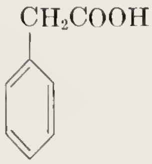


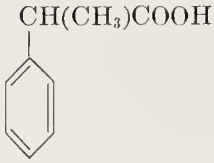
Δ^1-Cyclohexenyleneacetic acid
(Active)

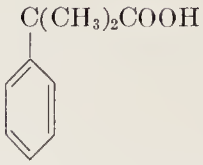
Several theories have been put forward to explain the importance of unsaturation in this connection. The view of Commoner and Thimann (23) that a reversible oxidation-reduction property of the double bonds might enable auxins to act as respiratory catalysts was disputed by Veldstra (119) who put forward evidence to show that the degree of unsaturation influenced the surface activity of the molecule, which he considered to be one of the factors determining growth-regulating activity (see also 118).

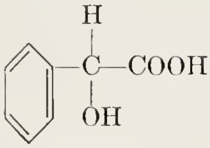
C. THE SIDE CHAIN

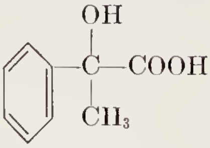
All aryl- and aryloxyacetic acids and their higher homologues possess the type of side chain considered necessary for activity by Koepfli *et al.* (63). Aryloxy acids were not investigated by these workers, but the growth-regulating activity in the pea test of compounds obtained by substituting groups into the side chain of phenylacetic acid was reported. Thus, the activities of phenylacetic, α-phenylpropionic, and α-phenylisobutyric acids were rated 2, 6, and 0%, respectively, of that shown by indole-3-acetic acid. Mandelic, atrolactic, and tropic acids were inactive. Koepfli *et al.* pointed out that their results did not warrant any conclusions with regard to the effects of substituents in the side chain (63).

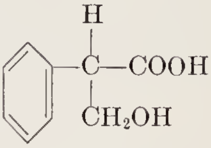

Phenylacetic acid
(Active)


α-Phenylpropionic acid
(Active)

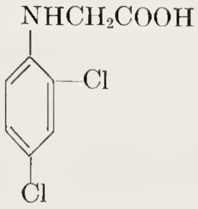

α-Phenylisobutyric acid
(Inactive)

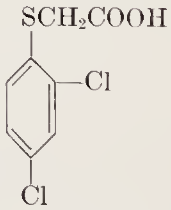

Mandelic acid
(Inactive)


Atrolactic acid
(Inactive)


Tropic acid
(Inactive)

The demonstration by Irvine (54) that 2-naphthoxyacetic acid possessed growth-promoting properties showed that the presence of an oxygen bridging group between ring and side chain is consistent with activity. The effect of replacing this oxygen atom in 2-naphthoxyacetic acid by —S— or —NH— was studied by Walker (137), who found these compounds inactive for setting tomatoes. *N*-2,4-Dichlorophenylglycine, however, is highly active in the pea test (26,125), as are also about half of the sixty phenylthioalkanecarboxylic acids investigated by Fawcett, Wain, and Wightman (30). It is therefore clear that a side chain containing —NH— or —S— can fulfill the requirements for this type of growth-regulating activity (see also 59).

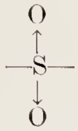

N-2,4-Dichlorophenylglycine
(Active)


2,4-Dichlorophenylthioacetic acid
(Active)

There is now much evidence to show that aryloxy acids are in general more active than their arylthio analogues (30). When the sulfur bridging group (—S—) is oxidized to sulfoxide

$$\underset{\substack{\downarrow\\ \mathrm{O}}}{-\mathrm{S}-}$$

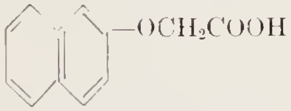
activity decreases considerably and with further oxidation to sulfone



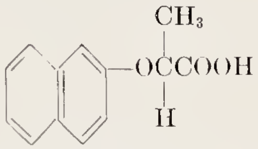
$$-\overset{\overset{O}{\uparrow}}{\underset{\underset{O}{\downarrow}}{S}}-$$

it disappears completely (24).

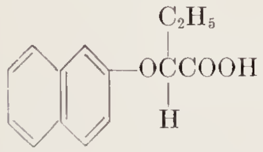
In growth substances derived from indole and naphthalene, the position of the side chain has been shown to be important. Thus, indole-3-acetic acid is active, whereas indole-2-acetic acid has low activity (67). Similarly, 1-naphthylacetic is much less active than 2-naphthylacetic acid (148), and 2-naphthoxyacetic acid is active, while 1-naphthoxyacetic acid has negligible activity (119). The effect on activity of substituting alkyl groups into the side chain of aryloxy- and arylthio-alkanecarboxylic acids has received considerable attention (26,30,89,91) following an observation in the writer's laboratory (137) that, although 2-naphthoxyacetic, α-(2-naphthoxy)-propionic and butyric acids were highly active in the tomato parthenocarpy test, α-(2-naphthoxy)isobutyric acid was completely inactive.



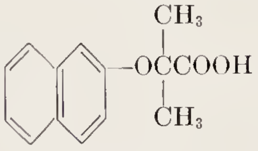
2-Naphthoxyacetic acid
(Active)



α-(2-Naphthoxy)propionic acid
(Active)



α-(2-Naphthoxy)butyric acid
(Active)



α-(2-Naphthoxy)isobutyric acid
(Inactive)

Some fifteen series of aryloxyacetic, -propionic, -butyric, and -isobutyric acids and the corresponding arylthio analogues have since been examined in a range of tests (30), and it has been found that in most cases only the acetic, propionic, and butyric derivatives possessed the capacity to induce cell elongation. Although a small number of the isobutyric acids exhibited some activity, the results in general indicated that molecular structure in the α-aryloxyisobutyric acids is not consistent with the type of growth-regulating activity which depends mainly upon a cell elongation response. Now the isobutyric acid derivatives differ from the corresponding acetic, propionic, and *n*-butyric derivatives in that the carbon atom adjacent to the carboxyl group carries no hydrogen atoms. These considerations led to the suggestion that, at least for types of activity depending on cell elongation, the presence of a hydrogen atom on the carbon *alpha* to the carboxyl group is necessary (89,91). This so-called α-hydrogen effect will be referred to again later when theories on the mode of action of growth substances are discussed. In the meantime, it is of interest to note that certain other types of compound appear to require an α-hydrogen atom in order to show activity as a growth substance. Thus, for example, whereas 1-naphthoic and 3,4-dihydro-1-naphthoic acids, possessing no hydrogen atoms on

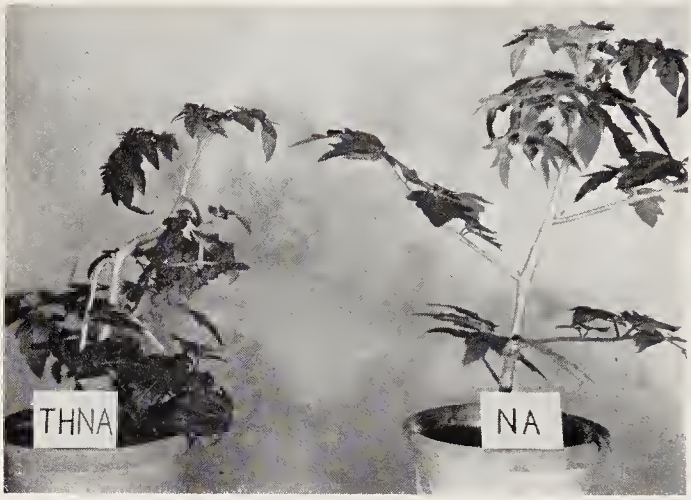
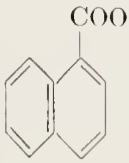
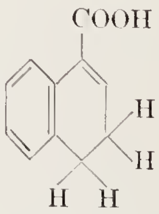


Fig. 3. Tomato leaf epinasty test. Plant *on left* has been treated with the active substance 1,2,3,4-tetrahydro-1-naphthoic acid (THNA); plant *on right* with 1-naphthoic acid (NA), which is inactive.



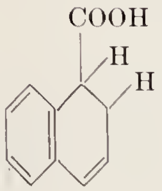
1-Naphthoic acid



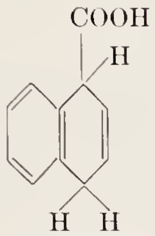
3,4-Dihydro-1-naphthoic acid

(Low activity)

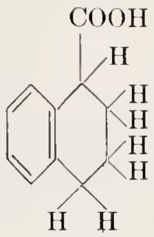
the carbon adjacent to the —COOH group, are practically inactive in the pea curvature and *Avena* cylinder tests, the 1,2- and 1,4-dihydro and the 1,2,3,4-tetrahydro derivatives are highly active, (53,58,126,128) (Fig. 3). Again, fluorene-9-carboxylic acid is active in the pea curvature and tomato leaf epinasty tests. Substitution of the hydrogen in the 9- position by methyl, however, leads to a complete loss of activity (128).



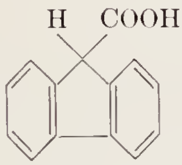
1,2-Dihydro-1-naphthoic acid



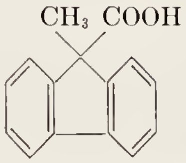
1,4-Dihydro-1-naphthoic acid (Highly active)



1,2,3,4-Tetrahydro-1-naphthoic acid

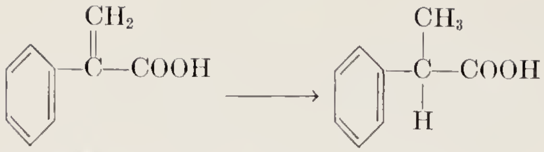


Fluorene-9-carboxylic acid (Active)



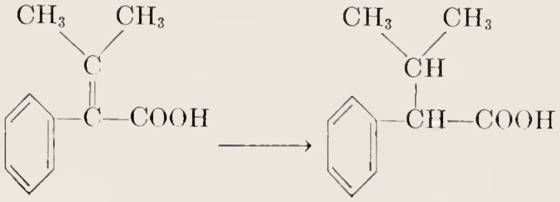
9-Methyl-fluorene-9-carboxylic acid (Inactive)

All these findings are consistent with the α-hydrogen hypothesis, though, as has been pointed out by Veldstra, a number of α-alkylidenephenylacetic acids are active, and no α-hydrogen atom is present in these molecules (124). Furthermore, α-(indole-3)isobutyric acid is also active (30). The possibility has been raised, however, that some of these compounds are changed within plant tissues to yield traces of more active substances which are responsible for the activity shown (30). Thus, the production of the α-propionic acid derivative by demethylation of the isobutyric acid could lead to a growth-promoting response; and, since some of these propionic derivatives are extremely active, only a slight degree of conversion would be necessary in these cases (30). Again, α-alkylidenephenylacetic acids could yield such propionic acids by reduction within the tissues:

 (1)

α-Methylenephenylacetic acid → α-Phenylpropionic acid

It has been pointed out by Veldstra (124), however, that such reduction cannot explain the activity of α-propylidinephenylacetic acid, for the product of such a reaction would be α-isopropylphenylacetic acid, which is an auxin antagonist.

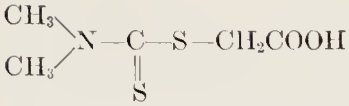
 (2)

α-Propylidinephenylacetic acid → α-Isopropylphenylacetic acid

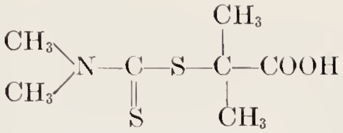
The fact that a number of benzoic and naphthoic acids can induce plant growth responses shows clearly that an α-hydrogen atom is not an essential requirement for all types of active compound. The hypothesis has proved useful, however, in the studies on mode of action of phenoxy acids to be described in Section III.F; and, as already shown, there is evidence that the requirement operates in the arylalkanecarboxylic acids.

A further demonstration of the α-hydrogen effect arose from the important discovery of van der Kerk and his coworkers (61) that carboxymethyl dimethyldithiocarbamate and certain of its analogues can show high growth-promoting activity. Since these molecules do not possess an unsaturated ring system, it is clear that such a feature is not an absolute requirement for growth-regulating activity. Although other active carboxylic acids not possessing a ring structure are known, none possess high activity (31).

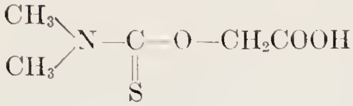
The compounds studied by van der Kerk *et al.* and later by other workers (31,124) are given below. It will be noted that the high activity of *S*-carboxymethyl dimethyldithiocarbamate is destroyed when the two α-hydrogen atoms are substituted by methyl groups (31,61,124).



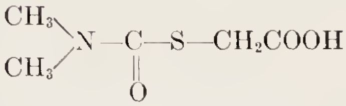
S-Carboxymethyl
dimethyldithiocarbamate
(Highly active)



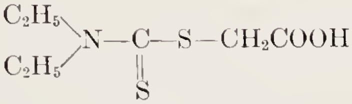
S-(β-Carboxyisopropyl)-
dimethyldithiocarbamate
(Inactive)



O-Carboxymethyl
dimethylthiocarbamate
(Highly active)



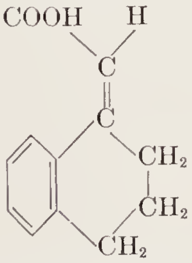
S-Carboxymethyl
dimethylthiocarbamate
(Active)



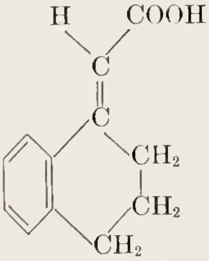
S-Carboxymethyl
diethyldithiocarbamate
(Active)

D. UNSATURATED SIDE CHAINS

Compounds containing a double bond in the side chain have been examined as plant growth regulators. Such substances can exist in *cis*- and *trans*- forms in which the spatial position of the carboxyl group with respect to the ring system is different. It is of considerable interest therefore, that, for example, only the *cis*- form of 1,2,3,4-tetrahydronaphthylidene-1-acetic acid possesses growth-promoting activity (119).



cis-1,2,3,4-Tetrahydro-
naphthylidene-1-acetic acid
(Active)

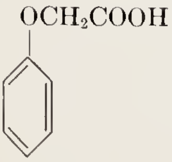


trans-1,2,3,4-Tetrahy-
dronaphthylidene-1-acetic acid
(Inactive)

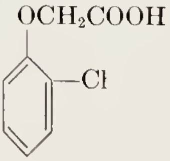
This behavior has been ascribed to the fact that the carboxyl group lies out of the plane of the ring only in the *cis*- acid (119). A structural requirement of this nature is considered by Veldstra to be a prerequisite for activity because it enables the molecule to become adsorbed at a cell boundary with the ring orientated in the lipoid and the polar —COOH group in the aqueous phase. Another example of this kind is cinnamic acid where again only the *cis*- acid is active (123). Evidence for the nonflat form of a number of *cis*-acids of this type has been obtained by ultraviolet spectra determinations (47).

E. SUBSTITUTION INTO THE RING SYSTEM OF PHENOXY ACIDS

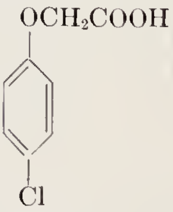
When chlorine is substituted into the 2-, 4-, or 2,4- positions of phenoxyacetic acid, the activity of the latter compound is greatly enhanced; on the other hand, 2,4,6-trichlorophenoxyacetic acid is almost inactive (109). Such considerations led Veldstra (121) to suggest that activity is determined by physicochemical considerations and particularly by a balance between the hydrophilic (COOH) and lipophilic portions of the molecule. It was considered that, in the chlorophenoxy series, the optimum balance is achieved in the 2,4-dichloro derivative, the weakly active 2,4,6- acid being overweighted on the lipophilic side. Such an explanation, however, is untenable, for not only are 2,3,5- and 3,4,5-trichlorophenoxyacetic acids active but the 2,4,5-trichloro and the 2,3,4,5-tetrachloro acids are highly active (71,118,134). Furthermore, 3,5-dichlorophenoxyacetic acid, in contrast to the 2,3-, 2,4-, 2,5-, and 3,4- acids, is almost inactive (71,134). Although isomers can differ in their physical properties,



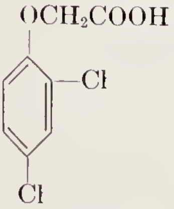
Phenoxyacetic acid
(Almost inactive)



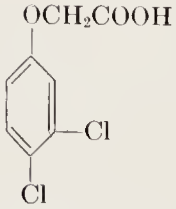
2-Chlorophenoxyacetic acid
(Active)



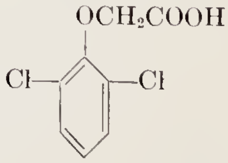
4-Chlorophenoxyacetic acid
(Highly active)



2,4-Dichlorophenoxyacetic acid
(Highly active)

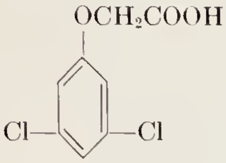


3,4-Dichlorophenoxyacetic acid
(Highly active)

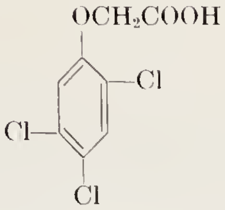


2,6-Dichlorophenoxyacetic acid
(Negligible activity)

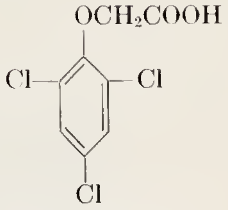
Veldstra's hypothesis does not adequately account for the marked differences in activity exhibited by positional isomers, a conclusion which he has also reached (123; see also 115).



3,5-Dichlorophenoxy acetic acid
(Negligible activity)



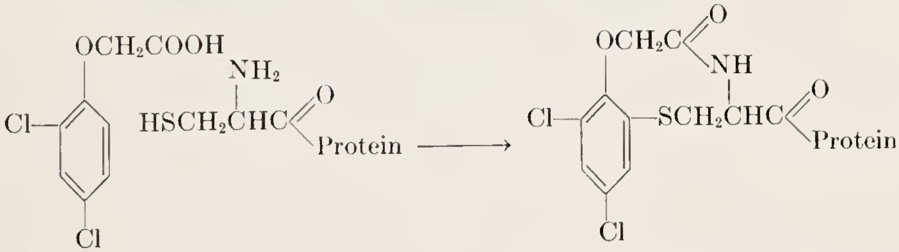
2,4,5-Trichlorophenoxyacetic acid
(Highly active)



2,4,6-Trichlorophenoxyacetic acid
(Negligible activity)

All the mono-, di-, and trichlorophenoxyacetic acids were examined as growth substances by Leaper and Bishop (71), and Wain and Wightman (134) assessed their activity in different tests. In both investigations, either low or negligible activity was found for all compounds which possessed substituents in the 2,6- or 3,5-positions of the ring (see also 115).

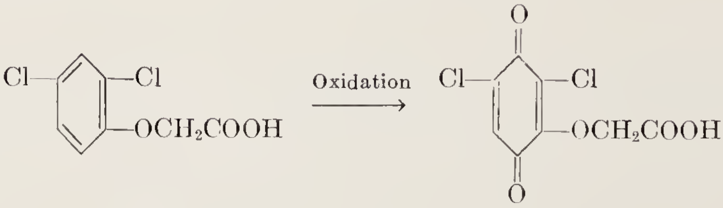
A number of theories have been put forward to account for the low activity which is usually associated with the presence of substituents in the 2- and 6-positions. Since these are the *ortho*-positions in the ring, it appeared that one such position must be free for activity to be shown and that this free *ortho*-position might be involved in some essential reaction within the plant whereby the growth response is induced. Such a chemical reaction was envisaged by Muir and his coworkers (45,46,82,83,85), who suggested that the reacting group within the cell might be the cysteinyl group of a protein which then undergoes a two-point *ortho*-reaction with the growth substances, as illustrated below with 2,4-dichlorophenoxyacetic acid.



(3)

This theory therefore demands a free *ortho*- position in the growth substance, if activity is to be shown. Thimann (115), while accepting the possibility that some cell constituent may react with the growth substance molecule, pointed out that ring positions other than the *ortho-* might be involved.

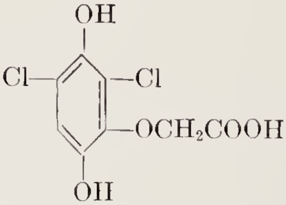
Leaper and Bishop (71), from a consideration of the growth-regulating activity of fifteen chlorophenoxyacetic acids, concluded that *para*-quinone formation might be an essential stage in the mode of action of such substances. Thus, 2,4-dichlorophenoxyacetic acid, for example, might first become oxidized as follows:



(4)

2,4-D

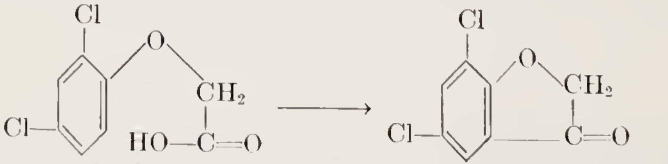
The quinone could then be reduced to the corresponding quinol:



The 2,4-D response, it is suggested, might arise from the production of one or both of these compounds within the cells.

It will be noted that the theory of Leaper and Bishop, like that of Muir and Hansch, involves a free *ortho-* position in the growth substance molecule.

Ring closure involving the free *ortho-* position to give a coumaranone derivative has also been suggested as an essential stage in the mode of action of phenoxy acids (57). The compound which would be formed in this way from 2,4-D, however, is inactive as a growth substance (99).



(5)

2,4-D

5,7-Dichlorocoumaran-3-one
(Inactive)

That hydroxylation of the ring might occur in the free *ortho-* position has also been considered as a possible factor in relation to plant growth-regulating activity (22,52).

There is now considerable evidence that di-*ortho-* substitution is not invariably associated with inactivity in the phenoxy acids.

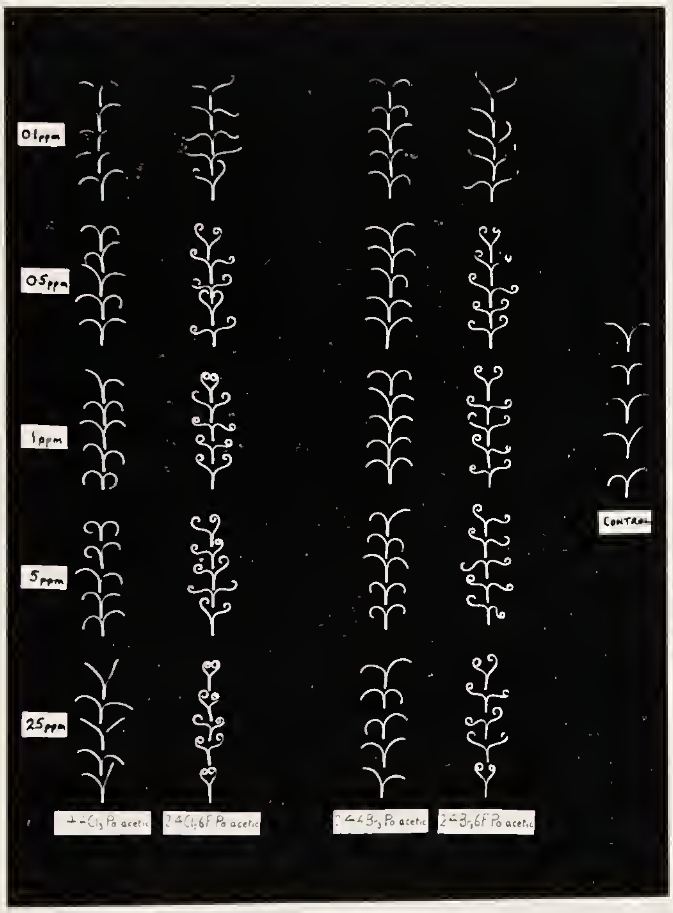
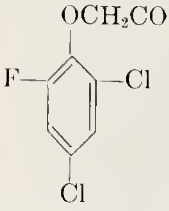
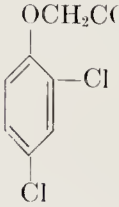

0.1 ppm
0.5 ppm
1 ppm
5 ppm
25 ppm
CONTROL
Cl3 Po acetic
2 4 Cl2 6F Po acetic
Br3 Po acetic
2 4 Br2 6F Po acetic

Fig. 4. Activity of 2,4,6-trisubstituted phenoxyacetic acids in the pea curvature test. High activity appears when fluorine replaces chlorine or bromine in the ortho position (118). Left to right: 2,4,6-trichloro-, 2,4-dichloro-6-fluoro-, 2,4,6-tribromo-, and 2,4-dibromo-6-fluoro-phenoxyacetic acids.

It has been established that 2,6-dichlorophenoxyacetic acid itself possesses some activity in the pea curvature test (115,118,134) and that other 2,6-substituted phenoxyacetic acids are also active (118). Furthermore, the introduction of a methyl or ethyl group into the side chain of 2,6-dichlorophenoxyacetic acid has been shown to lead to an increase in activity (88,118). The activity of all except two of a range of trisubstituted phenoxyacetic acids possessing groups in the 2-, 4-, and 6-positions was found by Toothill, Wain, and Wightman (118) to be negligible in three different tests, and with these ring systems, the α-substituted propionic acid was, in general, more active in the pea curvature test than the corresponding acetic acid. 2,4-Dichloro-6-fluoro- and 2,4-dibromo-6-fluorophenoxyacetic acids were the two acids which showed markedly different properties from those of the other 2,4,6-trisubstituted phenoxyacetic acids examined. These compounds exhibited very high activity in all tests. Thus, for example, the fluorodichloro derivative showed the same order of activity as 2,4-D, whereas 2,4,6-trichlorophenoxyacetic acid was almost inactive (118; see also 128) (Fig. 4 and Table I).


2,4-Dichloro-6-fluoro-phenoxyacetic acid
(Highly active)


2,4-D
(Highly active)

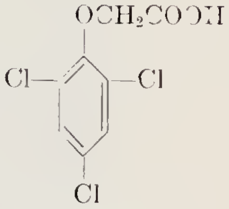

2,4,6-Trichlorophenoxy-acetic acid
(Almost inactive)

TABLE 1
Activity of 2,4,6-Substituted Phenoxyacetic Acids in the Wheat Straight Growth Test

Acid	Concentration in p.p.m.			
	1	5	25	100
2,4,6-Trichloro-	98	100	98	97
2,4,6-Tribromo-	99	101	98	98
2,4-Dichloro-6-fluoro-	138	143	145	126
2,4-Dibromo-6-fluoro-	110	116	132	117
Control	100	100	100	100

These fluoro compounds were prepared and examined because it was considered that the smaller diameter of the fluorine atom would permit a more free rotation of the side chain, such as occurs in the

2,4-dichlorophenoxyacetic acid molecule. With the 2,4,6-trichloro and -tribromo acids, such rotation is restricted (128) (Fig. 5). It is of interest in relation to these spatial considerations to note that Peters (95) has ascribed the toxicity of fluoroacetate in animal cells to the inability of the condensing enzyme of the citric acid cycle to distinguish between acetate and fluoroacetate, the dimensions of the fluorine and hydrogen atoms not being greatly different. If, however, dimensional factors alone account for the high activity of the 2,4-dichloro-6-fluoro- and 2,4-dibromo-6-fluorophenoxyacetic acids, then 2-fluoro-, 4-fluoro-, and 2,4-difluorophenoxyacetic acids should have low activity, since phenoxyacetic acid itself is only weakly active. This, however, is not the case. Further evidence that an explanation in terms of size of *ortho*- substituents is inadequate is provided by the finding that α-(2,4,6-trichlorophenoxy) propionic acid is active in the pea test, whereas α-(2,6-dichloro-4-methylphenoxy)propionic acid is inactive (118). Both these molecules, it will be noted, have chlorine atoms in the two *ortho*- positions.

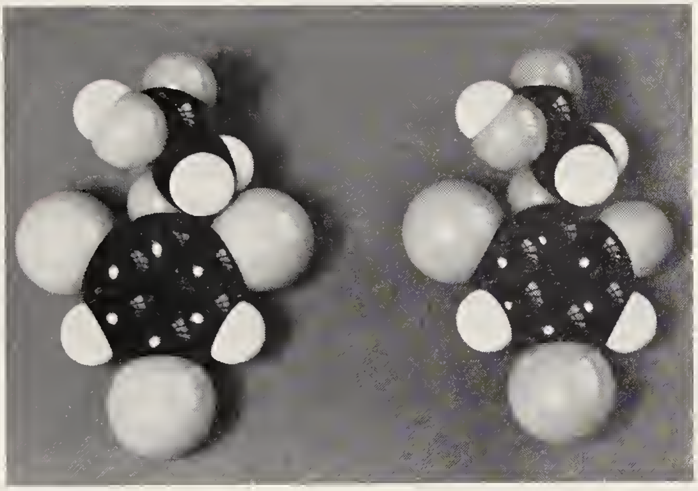
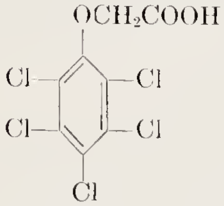


Fig. 5. Molecular models of 2,4,6-tribromo- (left) and 2,4-dibromo-6-fluorophenoxyacetic acids (right) (118).

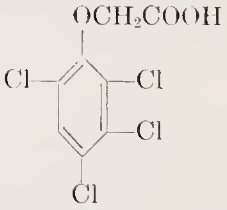
From the foregoing, it is clear that inactivity in phenoxy acids substituted in the 2,6- positions is not a general phenomenon and that theories such as those of Muir and Hansch, Leaper and Bishop, and others which demand a free *ortho*- position in the growth substance molecule are untenable.

Further work with 3,5-substituted phenoxy acids has recently been carried out (30,118), and the negligible activity of 3,5-dichlorophenoxyacetic acid, reported by earlier workers (71,115,134), has been confirmed. The 3,5-dimethyl and 3-ethyl-5-methyl derivatives were also found to be inactive (118). Evidence was obtained, however, that further substitution into the ring can lead to activity; thus 2,3,5- and 3,4,5-trichloro acids are active. On the other hand, unlike the 2,6-acids, no marked effect on activity was apparent when a methyl group was introduced into the side chain of acids possessing 3,5-substituents in the ring (118).

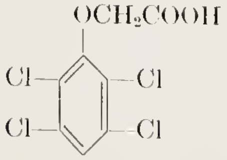
The possibility that growth-regulating properties in phenoxy acids are related to "activation" and "deactivation" of certain ring positions brought about by nuclear substituents has been considered by a number of workers (42,46,82,83,115). The fact that pentachlorophenoxyacetic acid is almost inactive would appear to indicate that at least one free "activated" ring position is necessary. Furthermore, the inactivity of 2,3,4,6-tetrachlorophenoxyacetic acid (114) and the corresponding 2,3,5,6-tetrachloro derivative (105) in the pea test suggests that the 4- and 5-positions are not involved in attachment, though the 6-position might be important, since 2,3,4,5-tetrachlorophenoxyacetic acid is active (118).



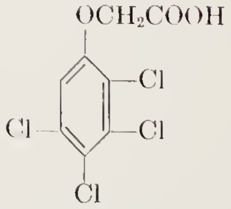
Pentachlorophenoxyacetic acid
(Inactive)



2,3,4,6-Tetrachlorophenoxyacetic acid
(Inactive)



2,3,5,6-Tetrachlorophenoxyacetic acid
(Inactive)



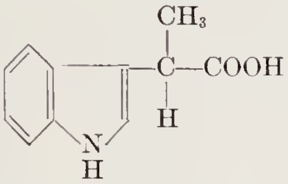
2,3,4,5-Tetrachlorophenoxyacetic acid
(Active)

As we have already seen, however, certain phenoxyacetic acids with both *ortho* positions occupied by substituent groups are appreciably active.

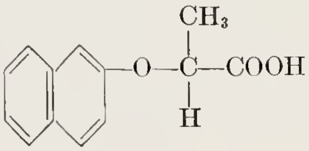
It would therefore appear that while some doubt remains on the importance of specific ring positions in relation to growth-regulating activity in the phenoxy acids at least one position must be free of substituents.

F. OPTICAL ISOMERS AND GROWTH-REGULATING ACTIVITY

When an alkyl group is substituted into the side chain of a growth substance derived from acetic acid, an asymmetric carbon is introduced and the resulting compound is optically active. Such a compound is capable of existing in two stereoisomeric (i.e., (+)- and (−)-) forms.



α-(Indole-3)propionic acid



α-(2-Naphthoxy)propionic acid

The first studies on the plant growth-regulating activity of stereoisomers were made by Kögl and Verkaaik (64,68) who found that (+)- α-(indole-3)propionic acid was thirty times more active than the (−)- isomer in the *Avena* curvature test. The two enantiomorphs were equally active, however, in the *Avena* straight growth test. These differences in relative activity were ascribed to differences in the ease with which the respective molecules moved within the tissues. In the curvature test it was assumed that the (−)- isomer was transported much less readily than the (+)- form, whereas under the conditions of the straight growth assay, where the coleoptile segments are immersed in the test solution, transport was regarded as being of less importance.

The first comprehensive study of stereoisomers in the aryloxy series was made by Smith and Wain (106), who showed that while the (+)- form of α-(2-naphthoxy)propionic acid is highly active in six different types of test, the (−)- form possesses negligible activity. In agreement with these findings Thimann (114) found that, in the pea test, the (+)- isomer of α-(2,4-dichlorophenoxy)propionic acid has approximately twice the activity of the racemic form, and Veldstra and Westeringh (126) found (−)-1,2,3,4-tetrahydro-1-naphthoic acid and (+)-α-allylphenylacetic acid to be highly active in the pea test, the corresponding enantiomorphs possessing only low activity (see also (58)). Again the (+)- and (−)- forms of α-(2,4-dichlorophenoxy)propionic and α-(2,4,5-trichlorophenoxy)propionic acids were examined in two tests by Smith, Wain, and Wightman (107), and it was shown that while the (+)- acids possessed high activity, that of the (−)- acids was negligible (Fig. 6). In similar studies with optical isomers of aryloxy acids made by Åberg (2), using root growth inhibition as the basis of the test method, both forms of some compounds were active, although only one isomer in each case was found to possess high activity.

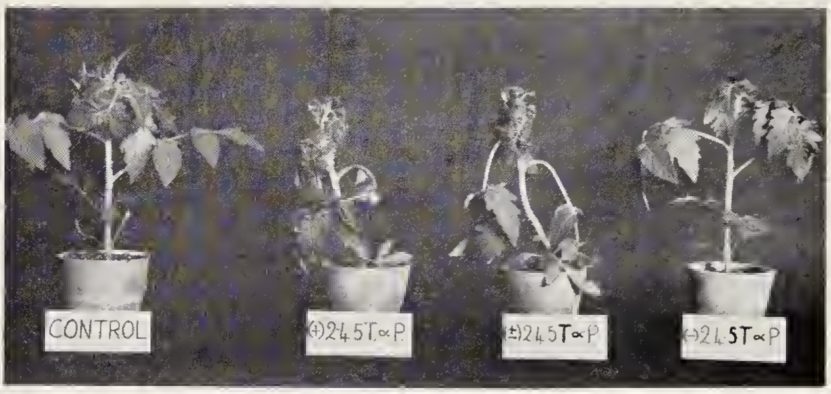


Fig. 6. Activity of the (+)-, (±)-, and (−)-forms of α-(2,4,5-trichlorophenoxy)-propionic acid in the tomato leaf epinasty test. Control plant *on left.*

Detailed investigations of a wide range of optically active growth substances have been made in Sweden by Fredga (33) and Matell (79,80). These workers also studied the formation of quasi-racemates as a method for determining the absolute configuration of the respective stereoisomers and in most cases were able to show that the more active form possessed the D- configuration (34,35,79). Indeed, so consistent were the results in these investigations that they raise the possibility of deducing the configuration of isomers from a consideration of their plant growth-regulating activity (32).

The observations that high activity in an optically active growth substance resides mainly in one of the stereoisomers suggest that this active isomer is capable of specific interaction with some asymmetric component present in the cell which is concerned in the production of the growth response.

This view has been developed by Wain and his coworkers (106,107), who have put forward a hypothesis on mode of action which explains these specific differences in activity of stereoisomers as well as the low activity which, as has been already noted, is usually associated with the α-aryloxyisobutyric acids. This theory assumes that growth-regulating activity in the aryloxy acids is dependent on the presence in the molecule of three "essential" groupings, namely, unsaturated ring system (R), α-hydrogen atom (H), and carboxyl group (A) and that when the molecule reaches the "primary site of action," which is regarded as some boundary surface within the cells, each of these groupings becomes associated with specifically placed cell receptor groupings (R′, H′, and A′). In this way a "three-point contact," so to speak, is made.

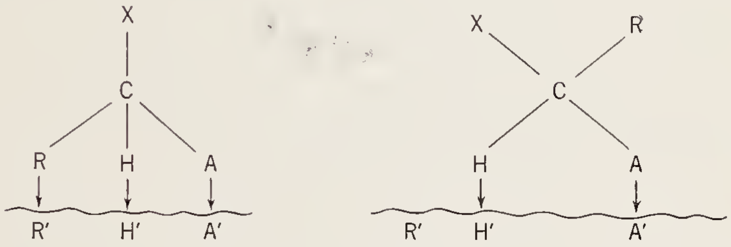


Fig. 7. *Left:* Three-point contact; active response. *Right:* Two-point contact; no response.

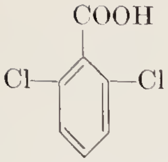
In optically active compounds, such an association between donor groups and acceptor sites is only possible with one stereoisomer, though the inactive enantiomorph can present two of the necessary groupings, as shown in Figure 7.

These considerations led Smith, Wain, and Wightman (107) to suggest the possibility that the inactive isomer, when present in large excess, might depress the activity of the active form by competition for the active receptor centers at site of action. Such competitive antagonism between stereoisomers has been clearly demonstrated (101,107), and in a later investigation a number of other molecules possessing two of the groupings considered essential were shown to act as growth substance antagonists both in the wheat straight growth and the pea tests (136).

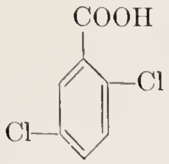
Other systematic studies on antagonism using root growth tests have been made by Erdtman and Burström and their collaborators in Sweden. This work has been summarized by Jönsson (56), who has also developed a theory on mode of action of growth substances which emphasizes the importance of the spatial shape of the molecules. In particular, he suggested that, for activity to be shown, the molecule must have an approximate planar structure with at least one side free from atoms other than hydrogen projecting from it and the carboxyl group must be in a certain position with respect to the ring (56). Schoot and Klaassens (98), however, have extended the three-point contact theory of Smith and Wain (106) to explain the activity of aryl- and aryloxyalkanecarboxylic acids. They consider that after attachment of the three essential groupings of the auxin molecule to the corresponding receptor units of the growth center, the —COOH group reacts to form a high-energy phosphate, whereupon a S_N2 reaction can take place at the α-carbon atom. It is pointed out by these workers that for this to occur, at least one α-hydrogen atom must be present in the molecule.

G. INFLUENCE ON ACTIVITY OF SIDE CHAIN LENGTH

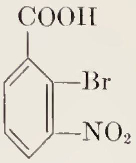
It is convenient here to consider briefly those compounds having a carboxyl group directly attached to the ring system. It has already been noted that certain substituted benzoic (11,145) and naphthoic (122,135) acids are active growth substances; 2,3,6-trichlorobenzoic acid, for example, is so highly active that it is finding important uses in selective weed control. Among a range of chlorinated and methyl-substituted benzoic acids studied by various workers, the 2,6-dichloro derivative was also shown to be active (122). Such findings (see also (83)) led to the idea that di-*ortho*- substitution in the benzoic acids was necessary for high activity. Veldstra (122), for example, has deduced from ultraviolet absorption spectra measurements that with the 2,6-substituted acids, steric inhibition of resonance occurs and in this way any tendency for the molecule to occur in a flat form is restricted. Thus, in these compounds, the dipole vector of the —COOH group will be at an angle with the plane of the ring system and so fulfill Veldstra's requirement for activity (119,120). No such simple explanation is valid, however, for not only is 2,5-dichlorobenzoic acid active (122) but 2-bromo-3-nitrobenzoic acid is also active in promoting a cell elonga-



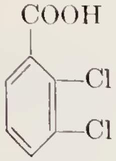
2,6-Dichlorobenzoic acid (Active)



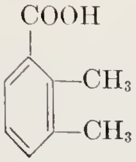
2,5-Dichlorobenzoic acid (Active)



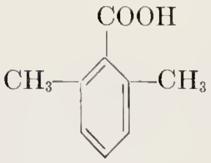
2-Bromo-3-nitrobenzoic acid (Active)



2,3-Dichlorobenzoic acid (Active)



2,3-Dimethylbenzoic acid (Active)

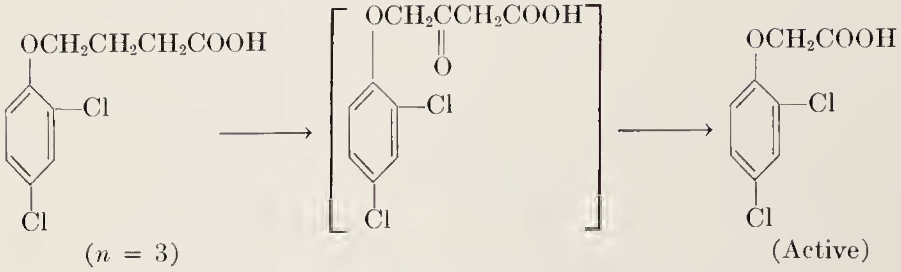


2,6-Dimethylbenzoic acid (Active)

tion response (145; see also 124). Furthermore, 2,3-dichloro- and 2,3,5-trichlorobenzoic acids are active in retarding root growth (55a) and the 2,3- isomer is also active in both the pea curvature and wheat straight growth tests (108). Some activity is shown by 2,3-dimethylbenzoic acid (107), and, although the corresponding 2,6-dimethyl acid is active (117,123), the 2,6-dibromo derivative is not (126a). In the light of these findings, some revision of the theories put forward (83,115,117,122) to explain the growth-regulating activity of benzoic acids in terms of di-*ortho*-substitution would appear to be necessary.

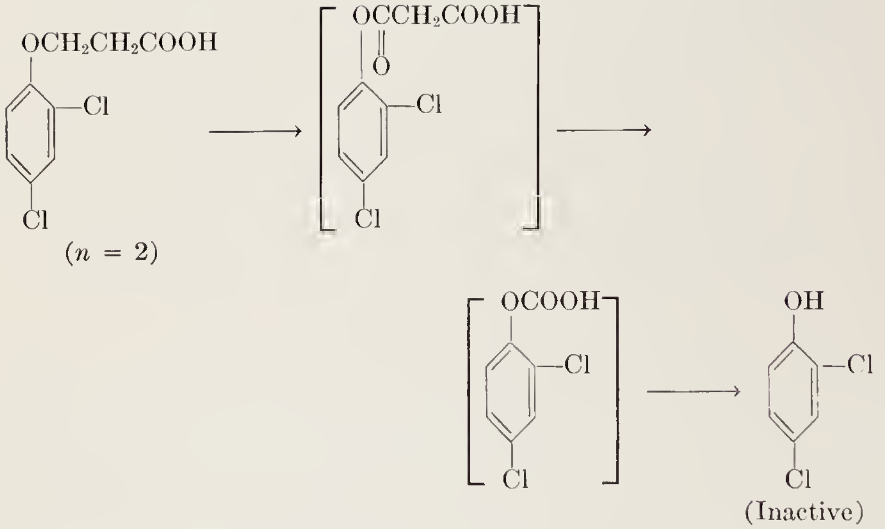
H. ACTIVITY IN HOMOLOGOUS SERIES

Several workers have demonstrated that the growth-promoting activity of ω-aryl- or ω-aryloxyalkanecarboxylic acids is related to side chain length and that an alternation in activity may operate as the series is ascended. Thus, Grace (40) examined the first five members of the ω-(1-naphthyl)alkanecarboxylic acid series ($C_{10}H_7$-$(CH_2)_nCOOH$) for their capacity to promote rooting of cuttings and showed that those compounds with an odd number of methylene (CH_2) groups in the side chain possessed an activity similar to that of 1-naphthylacetic acid, whereas the compounds with an even number of methylene groups had negligible activity. Synerholm and Zimmerman (110), using the tomato leaf epinasty test, found a similar alternation to occur with increasing length of side chain in a series of seven ω-(2,4-dichlorophenoxy)alkanecarboxylic acids ($Cl_2C_6H_3$-$O(CH_2)_nCOOH$). These workers suggested that degradation of the side chain by a β-oxidation mechanism could explain their results. By such means, acids with an odd number of side chain methylene groups might become converted to the active acetic homologue (e.g., equation 6), whereas those containing an even number might yield the parent phenol with no growth-regulating activity (e.g., equation 7).



$OCH_2CH_2CH_2COOH$
Cl
Cl
(n = 3)
OCH_2CCH_2COOH
O
Cl
Cl
OCH_2COOH
Cl
Cl
(Active)

(6)



OCH_2CH_2COOH
Cl
Cl
(n = 2)
$OCCH_2COOH$
O
Cl
Cl
$OCOOH$
Cl
Cl
OH
Cl
Cl
(Inactive)

(7)

Such an explanation is fully consistent with the alternation in activity which Fawcett, Ingram, and Wain (25) found to operate in the pea test for a series of ω-(4-chlorophenoxy)alkanecarboxylic acids and which Luckwill and Woodcock (77) found in the corresponding ω-(2-naphthoxy) series using the tomato parthenocarpy test. As early as 1904, Knoop (62) had suggested that fatty acids were degraded in the animal body by a process involving oxidation at the carbon atom in the β-position to the carboxyl group:

$$R.\overset{\beta}{C}H_2\,\vdots\,\overset{\alpha}{C}H_2\,COOH \longrightarrow R.COOH \qquad (8)$$

There is now overwhelming evidence that such β-oxidation does occur in the animal body (16) and that a number of enzymes which can only act on coenzyme A derivatives of these acids are involved (9,72,73). Chemical evidence that β-oxidation can operate within flax seedlings was obtained in the writer's laboratory using an homologous series of ω-phenoxy acids $C_6H_5O(CH_2)_nCOOH$, with $n = 1$ to 10, which were supplied to the seedlings through their roots (25).

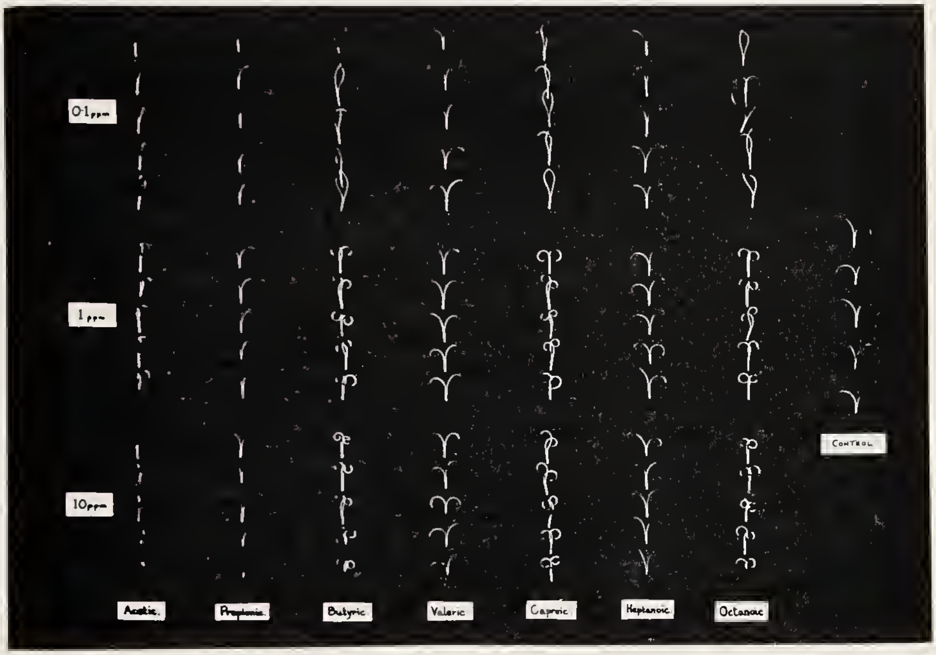


Fig. 8. Curvatures induced in the pea test by treatment with ω-(4-chlorophenoxy)-alkanecarboxylic acids at concentrations of 0.1, 1.0, and 10 p.p.m. with water controls *on right* (135).

Later Wain and Wightman (135) obtained further evidence that β-oxidation occurs in plants by studying the growth-regulating activity of members of homologous series of substituted phenoxy acids in a number of tests. Some twenty such series have now been investigated by Wain and his coworkers, each comprising at least the first six members from the acetic to the heptanoic derivative. In most instances, as, for example, with the ω-(4-chlorophenoxy)alkanecarboxylic acids, a typical alternation in growth-promoting activity was shown as the series was ascended both in the wheat straight growth and pea curvature tests (Fig. 8). This behavior, which is consistent with β-oxidation, was also shown, for example, in the corresponding ω-(2,4,5-trichlorophenoxy) series when examined in the wheat test. No such alternation, however, was shown by these compounds in the pea test, only the first member, 2,4,5-trichlorophenoxyacetic acid, being active (135) (Fig. 9 and Table II).

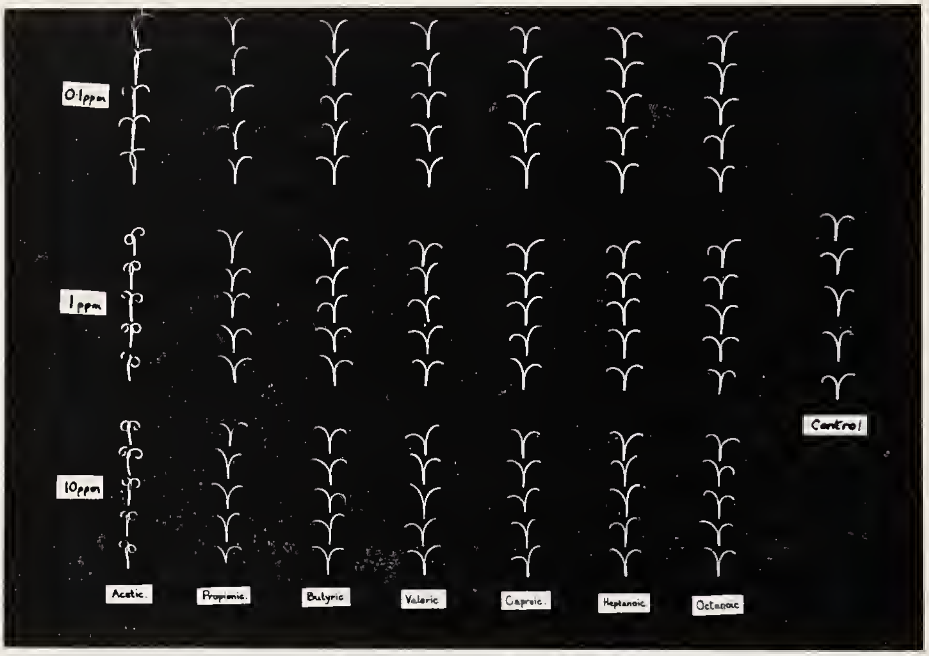


Fig. 9. Curvatures induced in the pea test by treatment with ω-(2,4,5-trichlorophenoxy)-alkanecarboxylic acids at concentrations of 0.1, 1.0, and 10 p.p.m. with water controls *on right* (135).

This discovery that γ-(2,4,5-trichlorophenoxy)butyric acid and its alternate higher homologues are active in one test and not in another indicated that specific β-oxidase enzyme systems may be present in different plant species. Evidence for this was obtained by direct experiment. Solutions of the 2,4,5-trichlorophenoxy homologues were first exposed to wheat coleoptile tissue and then examined in the pea curvature test. They now showed the typical alternation in activity indicating that the enzyme system present in the wheat tissue had degraded alternate homologues to the acetic derivative, active in the pea test (135). Further evidence that the enzymes in

TABLE II

Activity of Substituted ω-Phenoxyalkanecarboxylic Acids in Two Tests

Derivative	Number of methylene groups in side chain	4-Chloro series		2,4,5-Trichloro series	
		Wheat test	Pea test	Wheat test	Pea test
Acetic	1	Active	Active	Active	Active
Propionic	2	Inactive	Inactive	Inactive	Inactive
Butyric	3	Active	Active	Active	Inactive
Valeric	4	Inactive	Inactive	Inactive	Inactive
Caproic	5	Active	Active	Active	Inactive
Heptanoic	6	Inactive	Inactive	Inactive	Inactive
Octanoic	7	Active	Active	Active	Inactive

the wheat tissue were effecting β-oxidation of the side chain was obtained by treating wheat coleoptile tissue with solutions of γ-(2,4,5-trichlorophenoxy)butyric acid and demonstrating, by chromatographic methods, that conversion to the corresponding acetic acid had taken place. Results obtained with an homologous series of ω-(1-naphthyl) acids and studies on the effect of activity of substituting alkyl groups into the side chain of some of these compounds were also found to be explicable on the β-oxidation hypothesis (135).

All these results indicated that β-oxidation can occur in plants and that specific β-oxidase enzyme systems capable of degrading the side chain of a particular aryloxybutyric, -caproic, or -octanoic acid to the acetic derivative may be present in the tissues of one species and not in another. Such considerations indicated to the writer a new and fundamental principle upon which selective weed control might operate (130,131). Thus, one species of plant when treated with an aryloxybutyric -caproic, or -octanoic acid might be expected to degrade the compound to the highly active acetic acid and thus succumb to herbicidal action. Another plant species, however, not possessing the specific enzymes to break down these particular aryloxy acids to the same active end product, would remain unharmed. The results of experiments carried out along these lines were, in many cases, spectacular (129). Thus, for example, when Annual nettle (*Urtica urens*) or Canada thistle (*Cirsium arvense*) plants were sprayed with solutions of ω-(2-methyl-4-chlorophenoxy)alkanecarboxylic acids, the acetic, butyric, and caproic acids killed the plants, whereas those plants sprayed with the propionic, valeric, and heptanoic acids remained unaffected (Fig. 10). This type of behavior, however, was not obtained with all plant species. Thus, when clover or celery plants, for example, were sprayed with the above solutions, the acetic derivative produced drastic growth effects, but all the other homologues were almost completely inactive (Fig. 11). Such observations strongly supported the idea that a new type of selective weed control based on the plant's

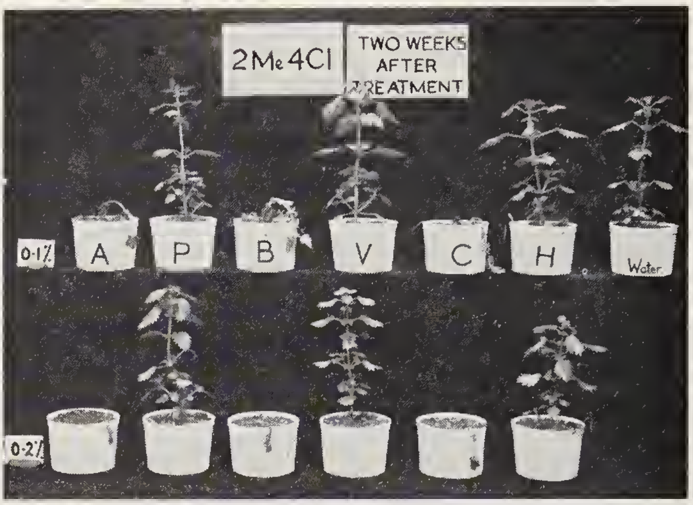

2Me4Cl
TWO WEEKS AFTER TREATMENT
0·1%
A
P
B
V
C
H
Water
0·2%

Fig. 10. Effect of spraying solutions of 2-methyl-(4-chlorophenoxy)-acetic, -propionic, -butyric, -valeric, -caproic, and -heptanoic acids on annual nettle plants (*Urtica urens*) (131).

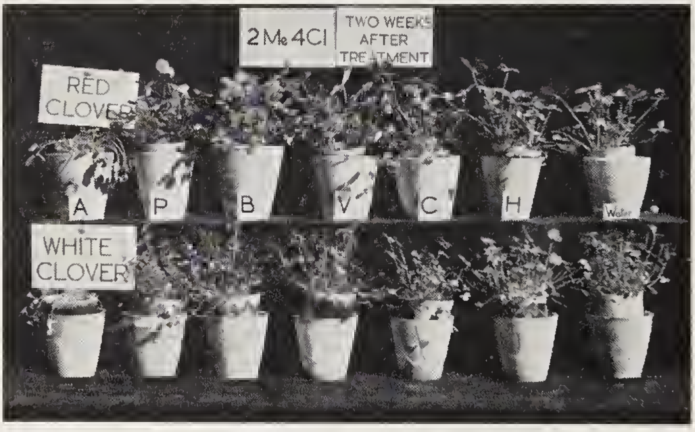
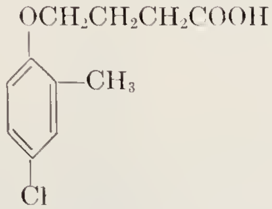
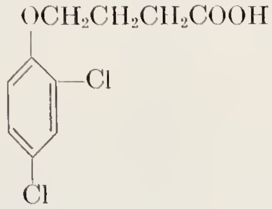

2Me4Cl
TWO WEEKS AFTER TREATMENT
RED CLOVER
A
P
B
V
C
H
Water
WHITE CLOVER

Fig. 11. Effect of spraying solutions of 2-methyl-(4-chlorophenoxy)-acetic, -propionic, -butyric, -valeric, -caproic, and -heptanoic acids on red clover (*Trifolium pratense*) and white clover (*T. repens*) (131).

own enzyme makeup was possible. Further work carried out on a range of crops and weeds has abundantly confirmed this (21,129), and already one of these materials, namely, γ-(2-methyl-4-chlorophenoxy)butyric acid (MCPB), is being widely used by farmers in Britain for controlling weeds in clover and peas (132).



γ-(2-Methyl-4-chlorophenoxy)-butyric acid
(MCPB)



γ-(2,4-Dichlorophenoxy)butyric acid
(2,4-DB)

Another of these compounds, γ-(2,4-dichlorophenoxy)butyric acid (2,4-DB), is also being developed as a selective herbicide for use on cereals and alfalfa. Both of these compounds are effective for killing a range of weeds in various crops at less than 2 pounds per acre, and they possess a further advantage over 2,4-D and MCPA

Fig. 12. Field of clover heavily infested with Canada thistle before spraying with MCPB (courtesy May and Baker Ltd.).

in that they can be safely applied to cereals at very early stages of growth (37). Since MCPB and 2,4-DB owe their activity to conversion to herbicide which would appear to occur only within the tissues of certain plants, the compounds possess a unique selectivity (Figs. 12 and 13). They are likely to prove useful for controlling weeds in crops all over the world and are already undergoing commercial development in a number of countries.

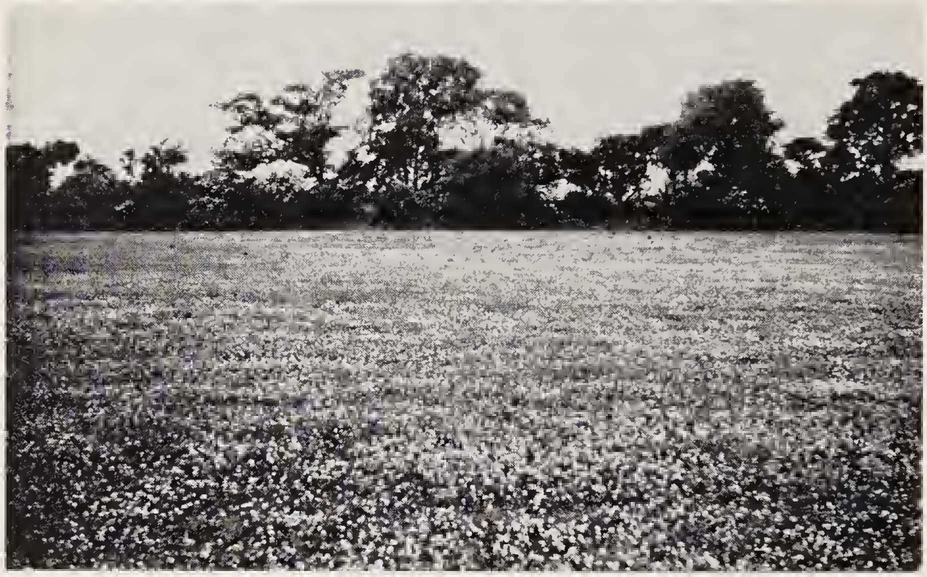


Fig. 13. Same field as Fig. 12 at harvest. Note thistles have been destroyed by the chemical treatment (courtesy May and Baker Ltd.).

I. AMIDE AND NITRILE DERIVATIVES OF ω-(ARYLOXY)ALKANECARBOXYLIC ACIDS

The first six members of the 2,4-dichlorophenoxy series, i.e., $Cl_2C_6H_3O(CH_2)_nCONH_2$ and $Cl_2C_6H_3O(CH_2)_nCN$, have been studied in the wheat straight growth and pea curvature tests by Wain and coworkers (28). In both tests the amide series showed an alternation in activity which can be explained in terms of hydrolysis to acid ($—CONH_2 \rightarrow —COOH$) followed by β-oxidation. With the nitriles, only the first member of the series (2,4-dichlorophenoxyacetonitrile) was active in the pea test. In the wheat test, however, all nitriles in the series were active (Fig. 14). Although hydrolysis ($—CN \rightarrow —COOH$) followed by β-oxidation would explain the activity of alternate homologues of 2,4-dichlorophenoxyacetonitrile (i.e., $n =$

3 and 5) in the wheat test, no such explanation is valid for the activity shown by other members of this series (n = 2, 4, and 6). In further experiments the 2,4-dichlorophenoxy series of acids, amides, and nitriles were exposed separately to wheat and pea stem tissues, and extracts were studied by paper chromatography (28). The chromatograms were divided transversely into ten equal segments, and each was assayed for growth-regulating activity by the wheat or pea test. In every instance where growth-regulating activity was shown by one of these compounds, chromatographic evidence was obtained that the compound could be metabolized in the plant tissue to yield 2,4-D. Thus, for example, in the acid and amide series, the alternation in activity associated with β-oxidation was accompanied by the appearance of 2,4-D when n was 1, 3, or 5.

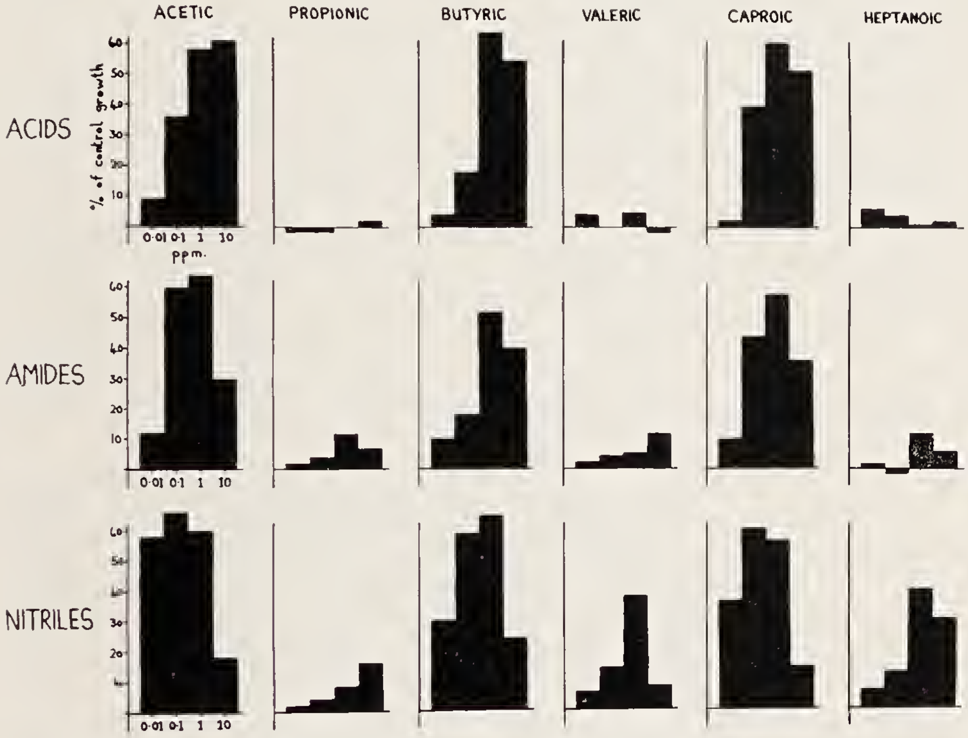


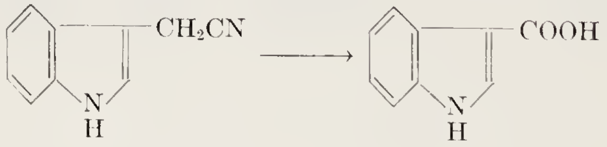
Fig. 14. Wheat straight growth test. Increases in length of wheat coleoptile segments following treatment with solutions of ω-(2,4-dichlorophenoxy)-alkanecarboxylic acids, amides, and nitriles (31).

In the wheat test the significant activity of all members of the nitrile series was accompanied in each case by the production of

2,4-D, the propionic derivative yielding the smallest amount. To explain the appearance of this acid from those members of the nitrile series where n (number of methylene groups) = 2, 4, and 6, the following mechanisms, in all of which a one-carbon fragment is lost at some stage, were put forward:

$$\begin{tikzcd}
\underset{(n = 2)}{\mathrm{Cl_2C_6H_3OCH_2CH_2CN}} \arrow[rrdd] & & \\
\underset{(n = 4)}{\mathrm{Cl_2C_6H_3OCH_2CH_2CH_2CH_2CN}} \arrow[d] & & \\
\mathrm{Cl_2C_6H_3OCH_2CH_2CH_2COOH} \arrow[rr, "\beta\text{-oxidation}"] & & \underset{(2,4\text{-D})}{\mathrm{Cl_2C_6H_3OCH_2COOH}} \\
\underset{(n = 6)}{\mathrm{Cl_2C_6H_3O(CH_2)_6CN}} \arrow[r] & \mathrm{Cl_2C_6H_3O(CH_2)_5COOH} \arrow[ru, "\beta\text{-oxidation}"'] &
\end{tikzcd} \quad (9)$$

These findings are of interest in relation to parallel studies on the metabolism of indole-3-acetonitrile in pea and wheat tissues (27,29) where by chromatographic methods the writer and coworkers have proved the following enzymic conversion to occur.



(10)

Indole-3-acetonitrile Indole-3-carboxylic acid

This degradation is precisely similar to that indicated in the above phenoxynitrile experiments (i.e., $—CH_2CN \rightarrow —COOH$) In both cases the α-methylene group becomes converted to carboxyl with the loss of one carbon fragment. The process may therefore be regarded as one of α-oxidation (27).

Although this α-oxidation of nitriles is a new reaction as far as the plant is concerned, there is evidence (3,94) that the $—CH_2CN$ grouping can be so degraded to carboxyl within the animal body. It would therefore appear that α-oxidation of nitriles, like β-oxidation of acids, may be a degradation mechanism which occurs not only in animal but also in plant biochemistry.

IV. Conclusion

It is clear from the work covered by this review that considerable progress has been made in relating chemical structure to plant growth-regulating activity in the twenty years or so since the organic chemist and biologist began their joint investigations within this field. The present tendency to study complete series of compounds from a number of different aspects is providing information for a rational approach to the understanding of the many and varied factors which operate in the auxin-induced growth response. The precise mode of action of growth-regulating substances, with the interplay of physical, stereochemical, enzymic, and other factors it involves, however, is far from understood. Nevertheless, the speculations arising from present-day investigations are, as has been shown, leading toward the discovery of new growth substances and to important agricultural developments.

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CHEMICAL STRUCTURE AND ACTIVITY OF DDT ANALOGUES WITH SPECIAL CONSIDERATION OF THEIR SPATIAL STRUCTURES*

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CONTENTS

I. Introduction 307
II. Criteria for Evaluating the Contact Insecticidal Activity of DDT Analogues 312
III. Considerations of Models of the DDT Analogues 317
  A. The Contactophore β,β,β-Trichloro-α,α-diphenylethane 319
  B. β,β,β-Trichloro-α,α-bis-(4-X-phenyl)-ethanes 321
  C. β,β,β-Trichloro-α,α-bis-(3-X-phenyl)-ethanes 323
  D. β,β,β-Trichloro-α,α-bis-(2-X-phenyl)-ethanes 325
  E. β,β-Dichloro-α,α-diphenylethylenes 325
  F. β,γ,γ-Trichloro-α,α-diphenylpropylenes 325
  G. β,β-Dichloro-α,α-diphenylethane and Its Derivatives 327
  H. α,β,β,β-Tetrachloro- and α,β,β-Trichloro-α,α-bis-(4-chlorophenyl)-ethanes 327
  I. The Stuart-Briegleb Models of DDT Analogues 327
  J. Results of the Model Investigations 330
IV. Configuration and Activity of DDT Analogues 330
V. Experimental Investigations of the Degree of Rotatability of the Molecular Components of a Few DDT Analogues 338
VI. Summary 348
References 349

I. Introduction

Since the discovery of the insecticidal properties of *p,p′*-DDT, or β,β,β-trichloro-α,α-bis-(4-chlorophenyl)-ethane (I), in the year 1939, several hundred analogous compounds ("DDT analogues") have been synthesized in different countries, and their insecticidal activity investigated (Tables I and II). The aim of these investigations was to discover DDT analogues that not only had outstanding contact insecticidal activity but also would surpass *p,p'*-DDT (I) with regard to activity and other qualities required of a good insecticide. One series of these investigations dealt predominantly or exclusively with fundamental principles of insecticides, especially, (*1*) obtaining evidence to explain the mode of action of DDT analogues, and (*2*) formulating principles by which the discovery of chemical pest control remedies, now predominantly empirical, could be placed on a systematic basis.

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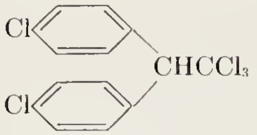
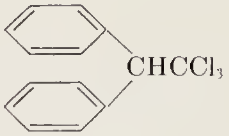
* Communication XIV of the series "Konstitution und Wirkung von Insektiziden." Communication XII, *Chem. Ber., 89,* 2702 (1956); Communication XI, *Chem. Ber., 89,* 2697 (1956); Communication X. *Z. angew. Entomol., 38,* 105 (1955); Communication IX, *Pflanzenschutz Ber., 13,* 97 (1954).



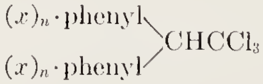
p,p'-DDT
(I)



(II)

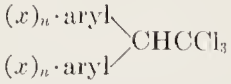
The modifications of the basic structure of the DDT series, β,β,β-trichloro-α,α-diphenylethane (II), have consisted essentially of:

1. Substitution in the benzene rings of the basic structure (II) in various ways, so that $(x)_n$ consists of the same or different substituents in each ring:



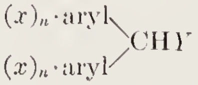
$$\left.\begin{array}{l}(x)_n\cdot\text{phenyl}\\(x)_n\cdot\text{phenyl}\end{array}\right\rangle\text{CHCCl}_3$$

2. Replacement of the benzene rings by other aromatic ring systems, such as furan, thiophene, naphthalene:

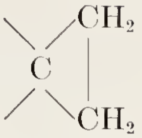


$$\left.\begin{array}{l}(x)_n\cdot\text{aryl}\\(x)_n\cdot\text{aryl}\end{array}\right\rangle\text{CHCCl}_3$$

3. Replacement of the CCl_3 group by other groups (Y), for example, $-CHCl_2$, $-CH_2Cl$, $-CCl{=}CCl_2$, $-CH_2-CHCl-CCl_2-CH_3$, $-CBr_3$, $-CHBr_2$, $-CH_2\cdot NO_2$, $-C(CH_3)_3$, $-COOH$, $-COCH_3$:



$$\left.\begin{array}{l}(x)_n\cdot\text{aryl}\\(x)_n\cdot\text{aryl}\end{array}\right\rangle\text{CH}Y$$

4. Replacement of the grouping CHY with  and others.

The first investigations of the chemical structure and activity of DDT-type compounds were carried out in Switzerland in the course of development of what are known today as the DDT contact insecticides. The first publications (exclusive of patents) of such investigations, however, were made by English authors (1943), and were followed by Swiss, American, Japanese, and German works, especially in the transition period 1946–47 (Table I).

The authors of all the works entered in Table I, examined and compared structures of the DDT analogues exclusively in terms of the usual structural formulas, that is, formulas obtained by projection of the molecule upon the plane of the paper. For the purposes of the experiments in synthesizing DDT analogues mentioned in Table I, the DDT analogues could be distinguished by means of simple structural formulas. However, there is another point of view which is important for characterization, namely, that one can simultaneously express how a substance is built or appears to be built according to the most recent knowledge. This latter point of view has been taken into account in comparative research on the activity of DDT analogues only in the last few years.

The first investigations of the constitution and activity in the DDT series in terms of spatial configuration were made in 1953 (Table II). It is significant that this development is based on the fact that in the remaining groups of contact insecticides of the class of halogen compounds, HCH and dienes, it was observed that the insecticidal activity of these compounds had a specific dependence on steric relationships. We think, for example, that the excellent insecticidal activity of the γ-isomer of 1,2,4,5,6-hexachlorocyclohexane (Gam mexane), m.p. 113°, is due to its chair configuration, e.e.e.a.a.a. Also *one* of the stereoisomers of 1,2,3,4,5,6,7,10,10-octachloro-4,7-methano-4,7,8,9-tetrahydrohydrindene (chlordane, M 410), and certainly the M 410-isomer of m.p. 104°, is marked by especially high insecticidal activity (33). To be sure, in these compounds, in contrast to Gammexane, the configuration is not yet completely determined.

TABLE I

Publications Concerning the Insecticidal Activity of DDT Analogues *without* Consideration of Their Spatial Configuration

Author(s) and title	Year	No. of analogues investigated	Ref.
England			
H. Martin, A. Stringer, R. L. Wain: The qualitative examination of insecticidal properties	1943	8	(19)
H. Martin, R. L. Wain: The qualitative examination of insecticidal properties	1944	15	(18)
O. Stephenson, W. A. Waters: Chemical investigations of the insecticide DDT and its analogues, II	1946	12	(48)
J. R. Busvine: Insecticidal tests with analogues of DDT	1946	40	(6,7)
Switzerland			
A. Mylius, H. Köchlin: Berichtigung zur Arbeit von P. Läuger, H. Martin, P. Müller (1944) "Über die Konstitution und toxische Wirkung von natürlichen und neuen synthetischen insektentötenden Stoffen"	1946	17	(25)
R. Domenjoz: Biologische Wirkung einiger DDT-Derivate	1946	16	(11)
P. Müller: Über Zusammenhänge zwischen Konstitution und insektizider Wirksamkeit, I	1946	52	(24)
United States			
E. H. Siegler, S. I. Gertler: Toxicity of diaryltrichloroethanes and dichloroethylenes to codling moth	1944	5	(45)
E. A. Prill, A. Hartzell, J. M. Arthur: Insecticidal activity of some alkoxy analogs of DDT	1945	5	(27)
E. A. Prill, M. E. Synerholm, A. Hartzell: Some compounds related to the insecticide DDT and their effectiveness against mosquito larvae and house flies	1946	42	(28)

TABLE I (*Continued*)

Author(s) and title	Year	No. of analogues investigated	Ref.
S. J. Cristol, H. L. Haller, A. W. Lindquist: Toxicity of DDT isomers to some insects affecting man	1946	3	(8)
C. C. Deonier, H. A. Jones, H. L. Haller, E. Hinchey, H. H. Incho: Mosquito larvicide tests	1946	38	(10)
H. L. Haller: Wartime development of insecticides	1947	28	(13)
G. T. Bottger, C. Levin, S. I. Gertler: Synthetic organic compounds as insecticides III	1947	5	(2,3)
R. L. Metcalf, F. A. Gunther: Mechanism of insecticidal action	1947	9	(21)
D. D. Questel, R. V. Connin: Investigations of spray for control of the European corn borer	1948	6	(30)
Canada			
M. D. Proverbs, F. O. Morrison: The relative insecticidal activities of DDT and related organic molecules	1947	12	(29)
Japan			
M. Hamada, M. Ohno, S. Takei: Studies on the DDT related compounds	1946	9	(14)
Germany			
R. Riemschneider, Konstitution und Wirkung von Insektiziden Mitt. I. u. II; Über die Konstitution kontakt-insektizider Substanzen	1947	70	(31,32)

As we progressed further, we found that a comparison of insecticidal activity of DDT analogues with configuration, i.e. spatial structure, yielded more information than could be obtained by the use of ordinary structural formulas, provided the following conditions were met:

1. The differences in spatial structures of the individual DDT analogues are ascertained, followed by construction and observation

TABLE II

Publications Concerning the Insecticidal Activity of DDT Analogues *with* Consideration of Their Spatial Configuration

Author(s) and title	Year	Ref.
United States		
E. F. Rogers, H. D. Brown, I. M. Rasmussen, R. H. Heal: The structure and toxicity of DDT insecticides	1953	(44)
England		
A. Stringer, D. Woodcock, E. J. Skerrett: Insecticidal activity and chemical constitution: Analogues and isosteres of DDT	1955	(49)
Germany		
R. Riemschneider: Stereochemische und toxikologische Untersuchungen in der DDT-Reihe	1953, 1954	(36,37) (38,39)
R. Riemschneider: Kritische Betrachtungen über Konfiguration und insektizide Wirkung von DDT-Analogen unter besonderer Berucksichtigung des sog. Trihedralisations-prinzips von Rogers und Mitarbeitern	1954	(40)
R. Riemschneider, W. Cohnen: Untersuchungen über den Grad der Drehbarkeit der Molekülkomponenten einiger DDT-Analoger	1956	(41)

of molecular models (Section III), and these observations are correlated with investigations of insecticidal activity (Section IV).

2. The criteria for the estimation of the contact insecticidal activity of DDT analogues presented in Section II are taken into consideration.

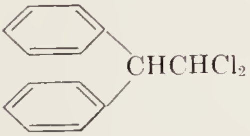
II. Criteria for Evaluating Contact Insecticidal Activity of DDT Analogues

The following criteria* are useful both to limit the number of DDT analogues from which insecticidal activity would be expected and to evaluate DDT analogues which are referred to in the literature as insecticidally active (it is assumed here that only the contact poison activity, and not any eventual or existing stomach poison activity, is being considered): (*1*) constitution of the contactophores and type, number, and position of positive as well as negative auxocontacting substituents in the contactophores (32); (*2*) molecular size; (*3*) lipoid solubility; and (*4*) melting point. The last three criteria are also generally useful for halogen compounds.

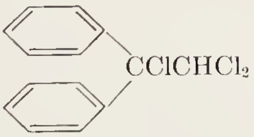
---

* These guide lines are naturally valid only for pronounced contact insecticides on the basis of their common absorption-mechanism. In this work "insecticidal activity" is understood as contact poisoning.

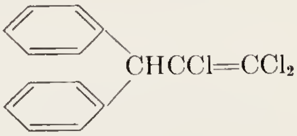
These criteria are here applied exclusively to the *entire molecule of the DDT analogue.* This opinion of the author is in opposition to that of Läuger and other theories, in which the *molecule of the DDT analogue is split into various components* (lipoid-solubilizing and toxic). If the concept of a toxic component is to be used at all, then in the opinion of the writer it should be taken very generally and it must be applied in terms of the basic framework of the DDT analogues. These and other reflections have led to the formulation of the concept of contactophores (14 or 15 carbon atoms (32)). Insecticidally active compounds can be derived from the following *contactophores:* β,β,β-trichloro-α,α-diphenylethane (II), β,β-dichloro-α,α-diphenylethane (III), α,β,β-trichloro-α,α-diphenylethane (IV), and β,γ,γ-trichloro-α,α-diphenylpropylene (V).



(III)

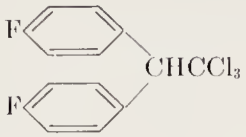


(IV)

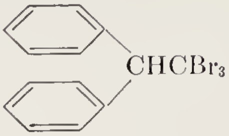


(V)

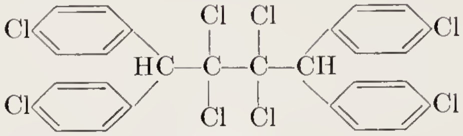
Introduction of substituents in fixed positions of the benzene ring influences the contact insecticidal action of these contactophores in a positive or negative sense. Positive *auxocontacts* can be: CH_3, C_2H_5, F, Cl, Br, CH_3O, C_2H_5O. Negative auxocontacts are: C_4H_9, C_6H_5, OH, COOH, NH_2, NO_2, among others. Simultaneous introduction of more than one auxocontact into the contactophores complicates the relationship. In p,p'-DFDT, β,β,β-trichloro-α,α-bis-(4-fluorophenyl)-ethane (VI), for example, both p-fluorine atoms are positive auxocontact elements of the contactophore β,β,β-trichloro-α,α-diphenylethane (II). With this concept of contactophore and auxocontact, which always work only in this way, we say the contact insecticidal activity of the *empirically discovered contactophores* can be influenced only through the introduction of the auxocontact building elements, in a positive or negative sense.



p,p'-DFDT
(VI)



(VII)



(VIII)

The *molecular weight* of all active DDT analogues lies between 270 and 450. These all show, likewise, good *lipoid-solubility* although, of course, no parallel exists between the degree of lipoid solubility and activity (Table III). *DDT analogues melting above 180°C.* which have good insecticidal activity are unknown. General establishment of these criteria and their use as guides to the discovery of new insecticides of the chlorinated hydrocarbon class have been discussed previously.* In support of this proposal the molecular weight, melting point, lipoid solubility, and positive auxocontacts of a few active DDT analogues are shown in Table IV.

TABLE III

Insecticidal Action and Lipoid Solubility of a Few DDT Analogues (11)

	Insecticidal activity		Solubility, g./100 ml.	
DDT analogue	*Pediculus*	*Musca*	Acetone	Olive oil
$(Cl^4\cdot C_6H_4)_2CH\cdot CCl_3$	Very active	Very active	21	9.6
$(C_6H_5)_2CH\cdot CCl_3$	Moderately active	Active	156	27
$Cl^2\cdot C_6H_4$ $>CH\cdot CCl_3$ $Cl^4\cdot C_6H_4$	Active	Active	77	11
$(CH_3{}^4\cdot C_6H_4)_2CH\cdot CCl_3$	Active	Very active	53	7.7

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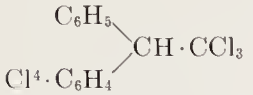
* Communication X of This Series (see footnotes on page 307).

TABLE IV
Molecular Weight, Melting Point, Lipoid Solubility, and Auxocontacts of a Few Active DDT Analogues

DDT Analogue	Mol. wt.	Melting point, °C.	Lipoid solubility, g./100 ml. acetone	Positive auxocontacts in the 4- and 4,4′-positions
Derived from the Contactophore β,β,β-Trichloro-α,α-diphenylethane				
 $(C_6H_5)(Cl^4 \cdot C_6H_4)CH \cdot CCl_3$	320	74		1 Cl
$(Cl^4.C_6H_4)_2CH \cdot CCl_3$	354.5	108	21	2 Cl
$(F^4 \cdot C_6H_4)_2CH \cdot CCl_3$	321.5	44	350	2 F
$(Br^4 \cdot C_6H_4)_2CH \cdot CCl_3$	443.5	140		2 Br
$(CH_3{}^4 \cdot C_6H_4)_2CH \cdot CCl_3$	313.5	89	53	2 CH_3
$(CH_3O^4 \cdot C_6H_4)_2CH \cdot CCl_3$	345.5	89	71	2 CH_3O
Derived from the Contactophore β,β-Dichloro-α,α-diphenylethane				
$(Cl^4 \cdot C_6H_4)_2CH \cdot CHCl_2$	320.0	111	50	2 Cl
$(F^4 \cdot C_6H_4)_2CH \cdot CHCl_2$	287.0	77		2 F

When all the four criteria are applied, the number of DDT analogues from which contact insecticidal activity may be expected is considerably reduced. For example, hydrophilic groups, such as OH and COOH, which decrease the lipoid solubility and raise the melting point, negatively influence the action of contactophores. Investigations with a large number of carboxyl and hydroxy compounds of the DDT series have confirmed this (Table V). Interchange of $—CBr_3$ for $—CCl_3$ raises the molecular weight too high in most cases, and only the basic substance β,β,β-tribromo-α,α-diphenylethane (VII) possesses activity. None of the DDT analogues nitrated in the aryl component has any insecticidal activity (NO_2 group is a negative auxocontact).

Applying these criteria to a few of the compounds designated in the literature as insecticidally active makes correction possible, as shown by the following two examples:

1. The strong contact insecticidal activity which different authors

TABLE V

Carboxyl and Hydroxy Compounds of the DDT Series
(Completely without Insecticidal Activity (35)

x	x'	Melting point, °C.
	β,β,β-Trichloro-α,α-bis-(x-x'-phenyl)-ethane	
4-COOH		141
4-$COOCH_3$		89
4-COOH	4′-CH_3	160
3-COOH-4-OH		186–187
3-$COOCH_3$-4-OH		116–117
3-COOH-4-OH	4′-CH_3	243
3-COOH-4-CH_3	3′-COOH-4′-CH_3	239–240
3-$COOCH_3$-4-CH_3	3′-$COOCH_3$-4′-CH_3	163.5
3-COOH-4-CH_3		162–163.5
3-COOH-4-OH	3′-COOH-4′-OH	291–292
3-$COOCH_3$-4-OH	3′-$COOCH_3$-4′-OH	201–202
3-CH_3-4-OH-5-COOH	3′-CH_3-4′-OH-5′-COOH	286
	β,β-Dichloro-α,α-bis-(x-x'-phenyl)-ethylene	
4-COOH		203
4-$COOCH_3$		64.5–65
4-COOH	4′-COOH	276–277
4-$COOCH_3$	4′-$COOCH_3$	188–189
3-COOH-4-OH	3′-COOH-4′-OH	295–297
3-$COOCH_3$-4-OH	3′-$COOCH_3$-4′-OH	120–122

have attributed to DDT-type condensation products of thiocyanobenzene and chloral seemed to be questionable because of the indicated melting point of 236°C. Adequate investigation showed the complete inactivity of this condensation product (43).

2. According to Bernimolin (1), 1,1,4,4-tetra-(*p*-chlorophenyl)-2,2,3,3-tetrachlorobutane (VIII), is about as active as *p,p′*-DDT to *Drosophila melanogaster*, although Deonier and coworkers (9) reported a few years previously that this compound had shown no activity to *Anopheles quadrimaculatus.* Although this contradiction could be based on the different susceptibilities of the test insects, the high molecular weight (638), the high melting point, and the low solubility of the chlorinated hydrocarbon in organic solvents argued against a high contact insecticidal activity. This led to our own research with this compound in the petri dish film test with *Drosophila melanogaster*, *Musca domestica*, *Calandra granaria*, and *Blatta orientalis* as test insects: VIII of m.p. 270°C., which was prepared from the catalytic hydrogenation of *p,p'*-DDT of m.p. 107.5–108.2°C., showed no activity even at high dosages (2 mg. per dish), while *p,p'*-DDT at 0.01 mg. per dish killed 100% of the *Drosophila* in less than 300 minutes. It is only conceivable that the compound prepared by Bernimolin (1) simulated activity because of contamination with DDT or that his substance had a different structure.

Both examples demonstrate the necessity for conducting toxicological investigations with the purest substances of exactly defined constitution and of known melting and boiling points. They also indicate that molecular size, degree of solubility, and melting point are of importance in evaluating the contact poison action of the halogenated hydrocarbons.

Even if all the above-mentioned criteria are considered many decisive questions remain unanswered with respect to constitution and effectiveness of the DDT group. For example, why do the position isomers of DDT analogues display such differing activity? Why is maximal activity always found in the 4,4'-positions? The molecular size of all members of an isomer group is the same, the melting point and lipoid solubilities are not basically different, and positive auxocontacts are present in all instances. According to these criteria, these single isomer groups should exhibit similar activity. Likewise, it is surprising that all the ethylene derivatives of the DDT series are practically inactive. Moreover, seemingly very slight alterations of the DDT molecule can result in complete ineffectiveness, even though all the criteria so far considered are still present. Molecular size, lipoid solubility, melting point, type of contactophore, and auxocontact are thus not sufficient to evaluate the contact insecticidal activity of the DDT analogues. These considerations resulted in a search for additional criteria for the activity of DDT analogues, and observations of molecular models were helpful (Section III). These had already been recognized as essential to the elucidation of several purely chemical questions relative to DDT analogues.

III. Considerations of Models of the DDT Analogues (38)

Molecular models according to Stuart and Stuart-Briegleb (4) were used in considering the spatial configuration of DDT analogues.

In drawing conclusions from consideration of such models, it is important to consider that they reproduce natural conditions only with limited precision. In particular, the hydrogen atom models are somewhat too large for many molecules, which may result in erroneous conclusions. Furthermore, only compounds with normal valence angles can be reproduced.

We will first consider the molecular models (Stuart models) of the most important contactophores, their 4,4′-, 3,3′-, and 2,2′-substitution products (for example, *p,p*′-DDT, *m,m*′-DDT, and *o,o*′-DDT), and several derivatives. The base of the Stuart models will be taken as the plane of reference. The different positions the components of the molecule can assume with respect to one another will, at first,

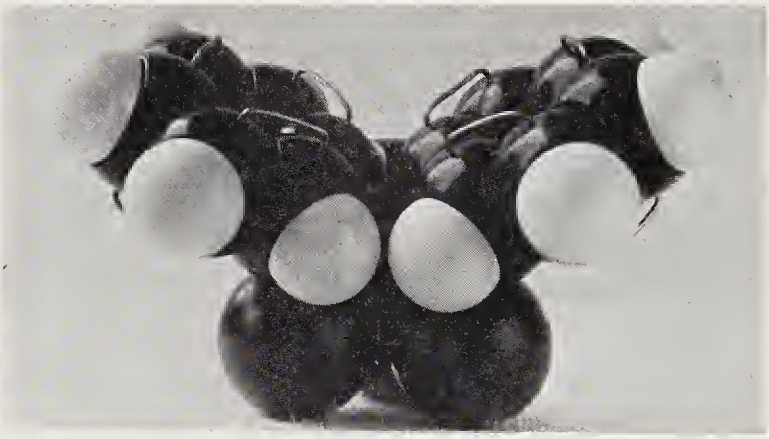


Figure 1

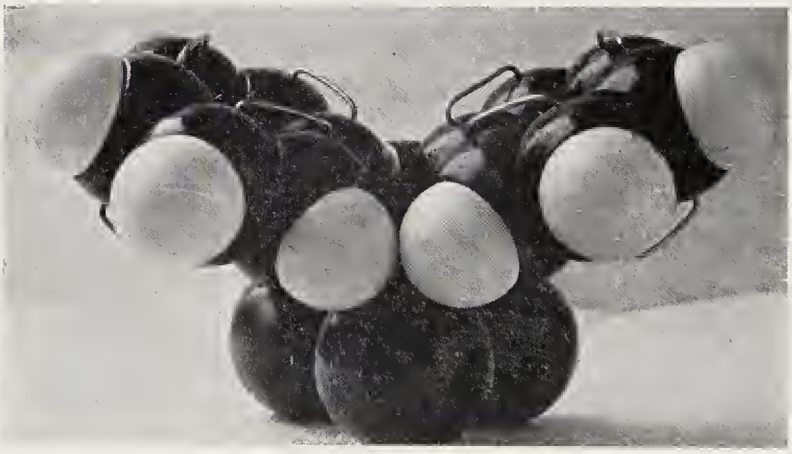


Figure 2

be described by means of pictures. This is a *static* approach. Subsequently, it will be discussed whether the different positions are interchangeable, i.e., whether the benzene rings and the CCl_3 group can rotate freely or whether they obstruct one another by reason of the spatial configuration of the molecule. This is the *dynamic* approach. The term "free rotatability" will be used only if all positions which the molecular components of a DDT analogue can assume in a model are interchangeable (38,39).

A. THE CONTACTOPHORE β,β,β-TRICHLORO-α,α-DIPHENYLETHANE (II)

In the Stuart model shown in Figure 1, the ethane axis, C_α–C_β, is perpendicular to the plane of reference. The planes of the two benzene rings are inclined at an angle of 30° from the plane of reference. The ethane axis is the symmetrical axis of the molecule because both benzene rings assume geometrically equal positions. The two *ortho* hydrogen atoms almost meet; thus they do not interfere with one another. However, the hydrogen atoms in ethane provide an energy threshold of approximately 3 kcal. which must be overcome in order to rotate the C–C bond, and the energy threshold is likely to be much higher in this case because of considerably greater proximity of the H atoms. If the CCl_3 group in Figure 1 is turned by 60° so that one Cl atom is directly under both of the *o*-H atoms

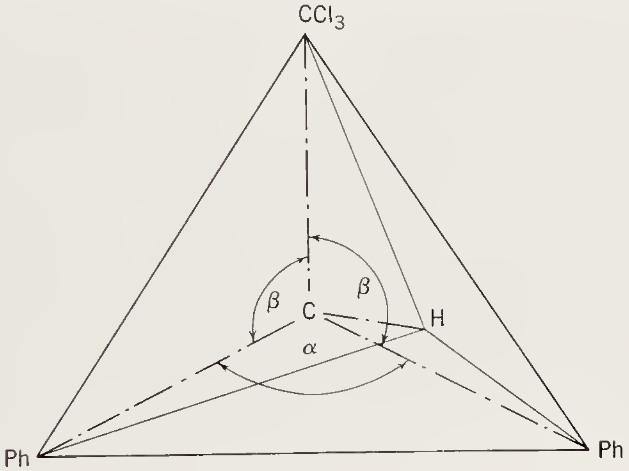


Fig. 3. Tetrahedral model of the α-C atom of β,β,β-trichlorodiphenylethane (II). α: angle between the C_1–C_4 axes of the phenyl nuclei; β: angle between C_1–C_4 axis of one phenyl nucleus and the CH—CCl_3 group (41,42).

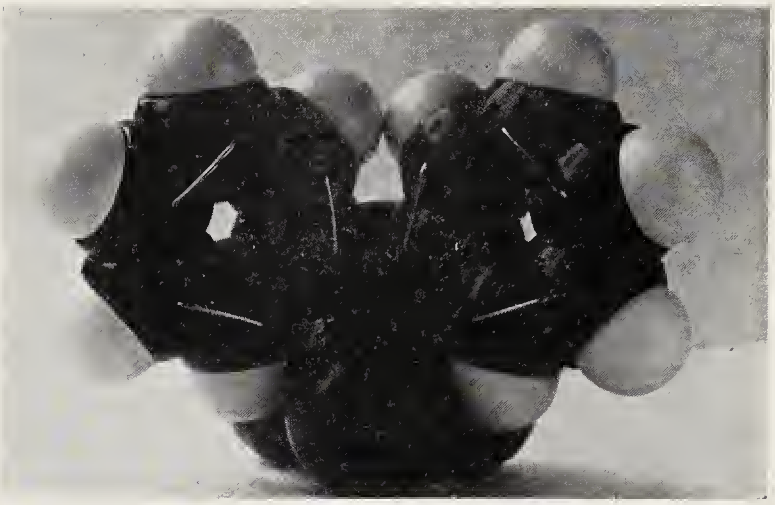


Figure 4

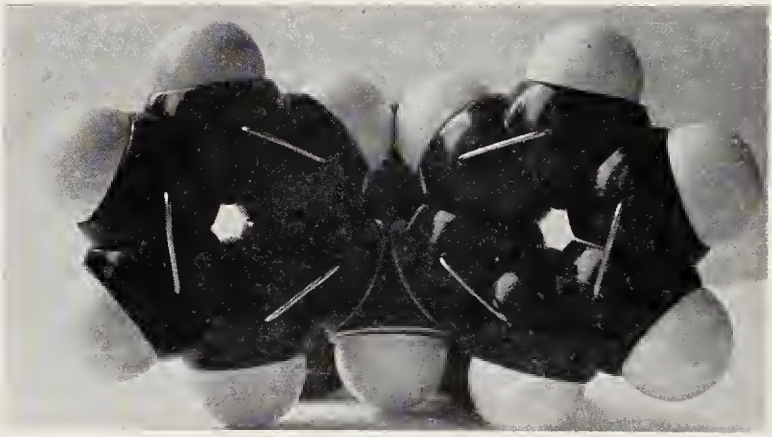


Figure 5

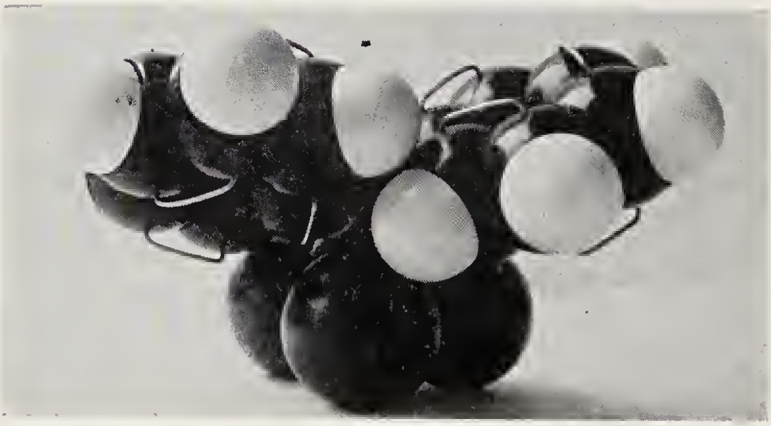


Figure 6

(Fig. 2), then these have to move upward and come closer together. This will result in a slight hindrance and consequently in a distortion of the valence angle, α, between the two benzene rings (Fig. 3). The hindrance becomes even more pronounced if both ring planes are turned by 60° (Fig. 4). The ethane axis and the ring planes then stand vertically on the reference plane. This arrangement can only be effected by considerable distortion of the valence angle, α (Fig. 3). The strongest hindrance is shown in Figure 5, where the ethane axis is parallel to the reference plane and the ring planes are almost vertical to the reference plane. The *o*-H atoms obstruct one another so strongly that an exactly vertical position of the ring planes can no longer be achieved. The increase in obstruction by the hydrogen atoms from Figure 1 through Figures 2–5 is reversible in each case through a small twist of the benzene rings against one another. These are then no longer geometrically equal positions, and the ethane axis is no longer the symmetrical axis (Fig. 6). All the described arrangements (Figs. 1 and 2–6) are interchangeable because the CCl_3 group also can rotate around the C–C axis (constellation series). The benzene rings can rotate freely around their own axis and in the course of their rotation interlock with the CCl_3 group. The individual components of the molecule (benzene rings and CCl_3 group) cannot rotate independently of one another, but they can rotate together if the degree of rotation freedom is activated.

B. β,β,β-TRICHLORO-α,α-BIS-(4-X-PHENYL)-ETHANES

The consideration of the Stuart models employed for the β,β,β-trichloro-α,α-diphenylethane (II) can be extended and applied to

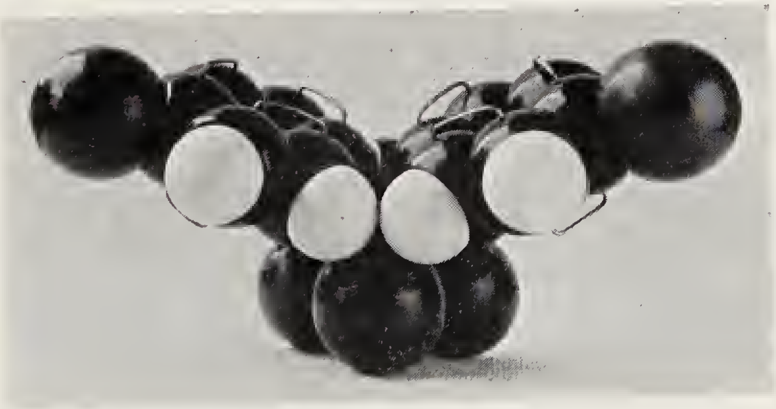


Figure 7

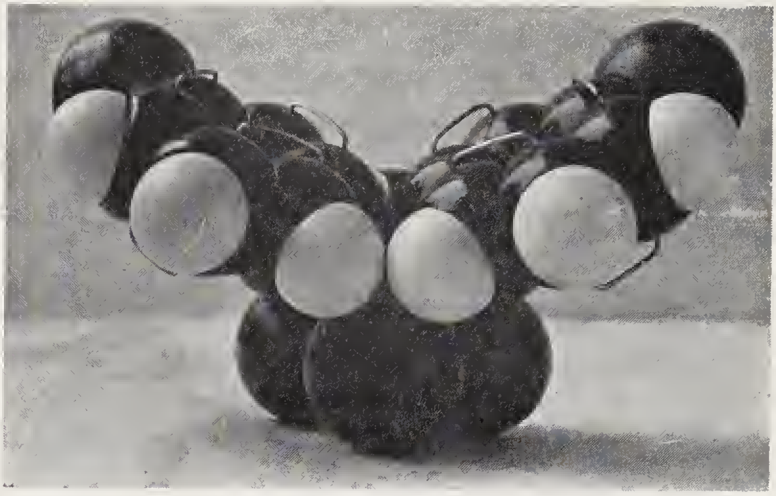


Figure 8

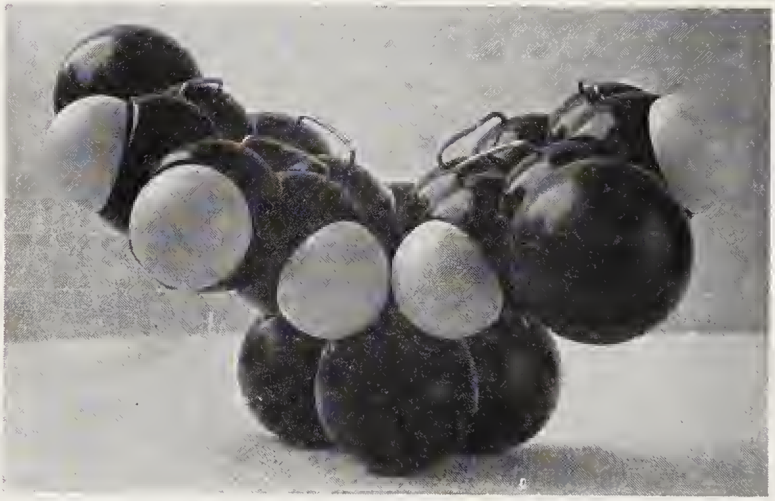


Figure 9

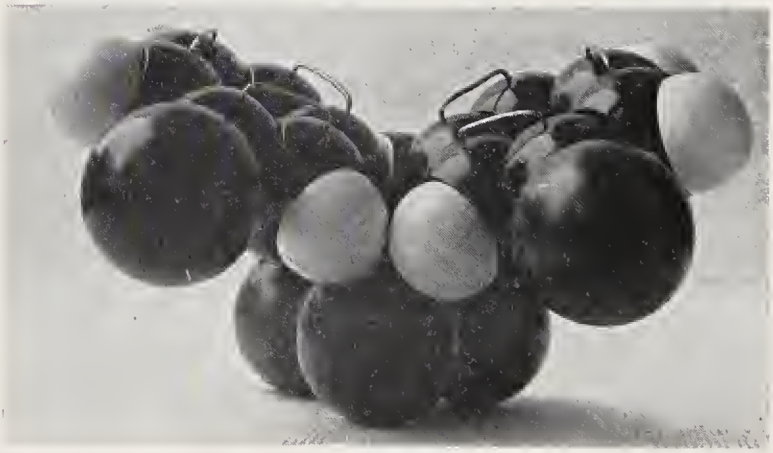
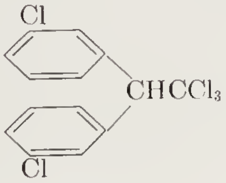


Figure 10

compounds substituted in the 4,4′-position. Substitution in the 4,4′-position does not alter the relation of possible hindrances of the molecular components to one another, as can be seen in Figure 7, which shows the *p,p′*-DDT molecule. But the distance of the *p*-substituents from the CCl_3 group changes somewhat if ring and CCl_3 group themselves turn. On account of the existing interatomic forces between the atoms of the substituents and the CCl_3 group, an energy minimum as well as an energy maximum can be distinguished at known positions. The maximum must be overcome in the course of the rotation; however, in the foregoing example the effect is very small, the distance being only insignificantly altered. The effect will vary with the nature of the substituent; for example, it is greater with Cl than with CH_3.

C. β,β,β-TRICHLORO-α,α-BIS-(3-X-PHENYL)-ETHANES

Two substituents introduced into the 3,3′-positions of the benzene rings of the contactophore β,β,β-trichloro-α,α-diphenylethane (II) do not alter the hindering properties of the basic substance. Figures 8–10 show the model of β,β,β-trichloro-α,α-bis-(3-chlorophenyl)-ethane (IX) arranged as in Figure 2. In Figure 8 both Cl substituents are away from the observer; in Figure 9 one is away and the other toward; in Figure 10 both are toward the observer. All other constellations shown in Figures 1 and 2–6 can also be achieved, and the ring has the same rotatability. This is limited only by the considerably higher energy differences that accompany rotation of the ring and the CCl_3 groups because the distance of the substituents from the CCl_3 group changes much more in the *meta*- than in the *para*-substituted compound. Certain constellations of the molecular components would be strongly favored.



m,m′-DDT
(IX)

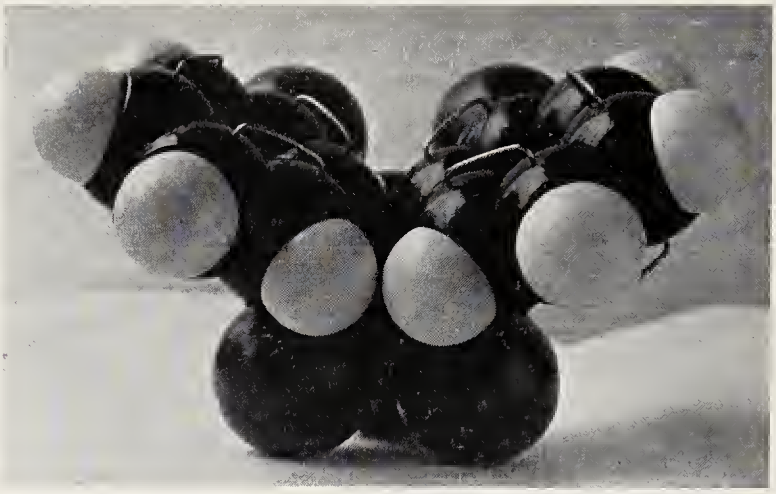


Figure 11

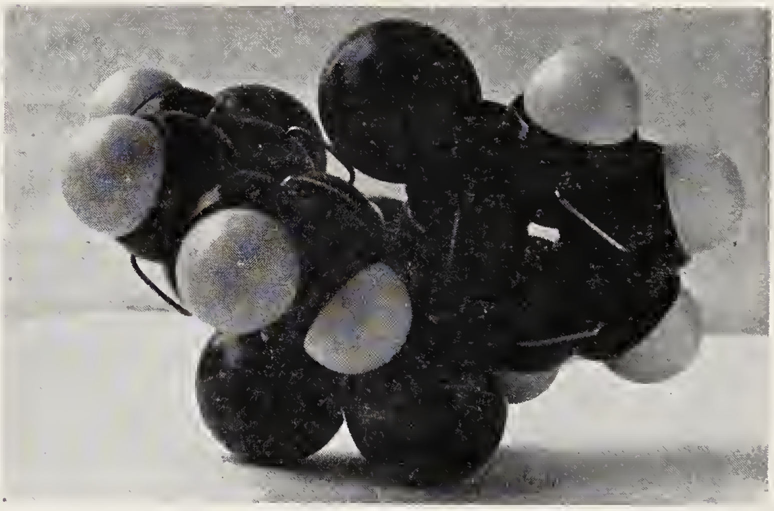


Figure 12

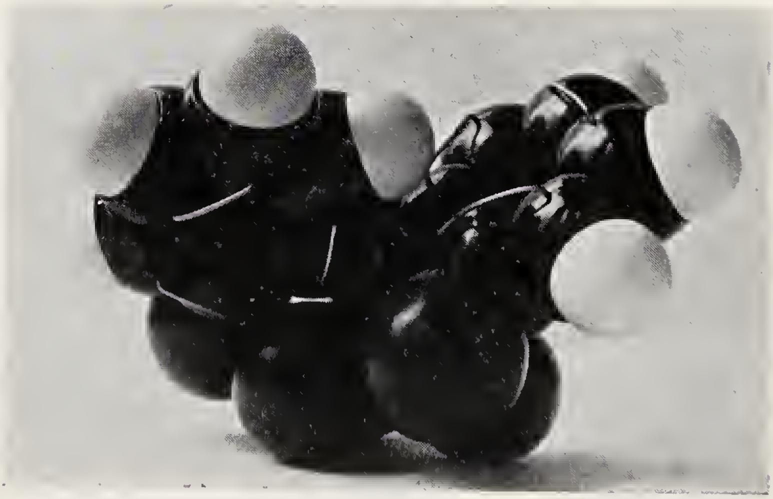


Figure 13

D. β,β,β-TRICHLORO-α,α-BIS-(2-X-PHENYL)-ETHANES

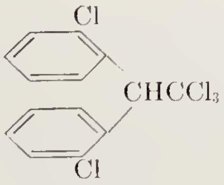
Completely different relationships are produced by 2,2′ substitution (Fig. 11). The constellation of the β,β,β-trichloro-α,α-diphenylethane (II) model shown in Figure 4 is not possible here. The arrangements in Figures 1 and 2 can be achieved only if both *o*-positioned substituents point backward. A free rotatability of both benzene rings which was indicated for the previously described Stuart models (toothed wheel type of interlocking with the CCl_3 group) likewise would be impossible. Other constellations of the β,β,β-trichloro-α,α-bis-(2-chlorophenyl)-ethanes (X) can be built which cannot be converted to one another, for example, Figures 12 and 13.

E. β,β-DICHLORO-α,α-DIPHENYLETHYLENES

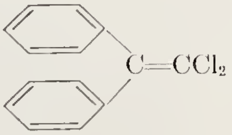
The rotatability of the benzene rings is hindered even in β,β-dichloro-α,α-diphenylethylene (XI), which is the basic substance of the series. The ethylene axis, C=C, stands perpendicular to the reference plane and is rigid. A rotation of the CCl_2 group is therefore not possible. The same is true, naturally, for all substitution products of the basic substance (e.g., β,β-dichloro-α,α-bis-(4-chlorophenyl)-ethylene (Fig. 14)).

F. β,γ,γ-TRICHLORO-α,α-DIPHENYLPROPYLENES

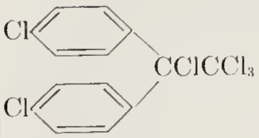
In β,γ,γ-trichloro-α,α-diphenylpropylene (V), in contrast to XI, both benzene rings and the trichloropropylene group are able to rotate freely (Figs. 15 and 16).



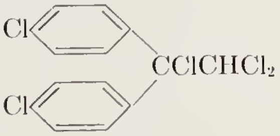
o,o′-DDT
(X)



(XI)



(XII)



(XIII)

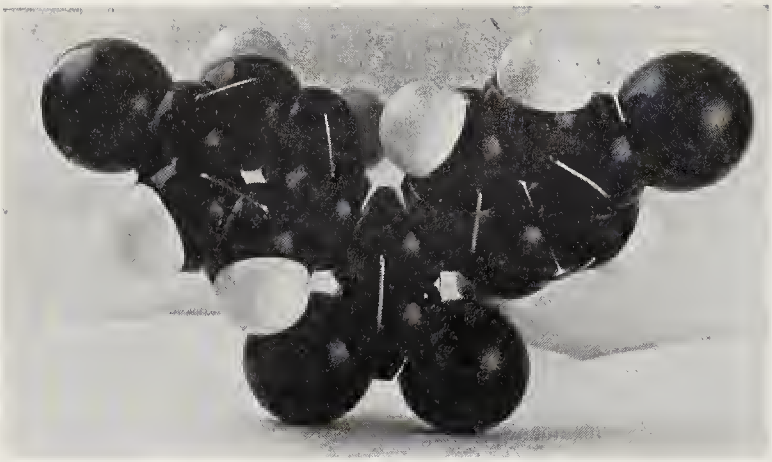


Figure 14

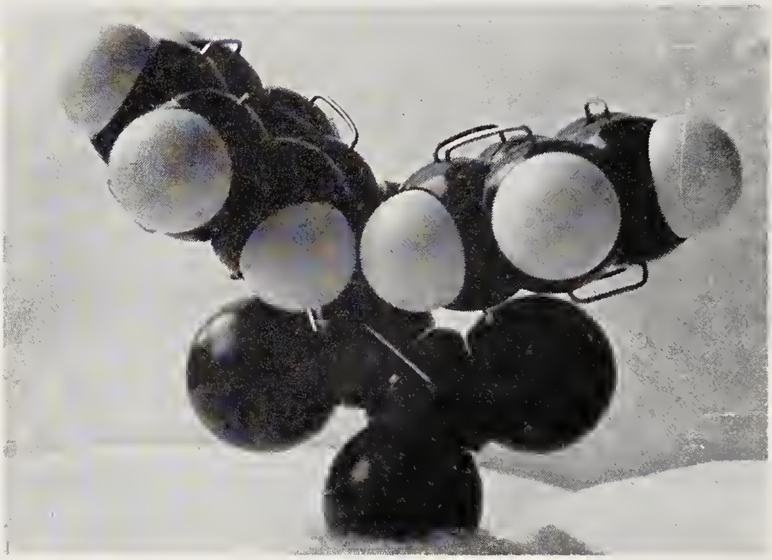


Figure 15

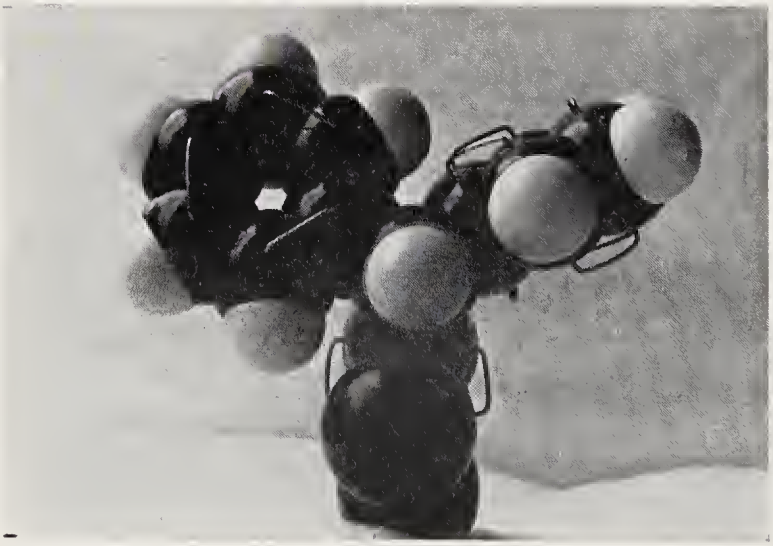


Figure 16

G. β,β-DICHLORO-α,α-DIPHENYLETHANE AND ITS DERIVATIVES

In β,β-dichloro-α,α-diphenylethane (III) and its substitution products, there is in all cases free rotatability of the benzene rings, even when the substitution occurs in the *o*-position. The $CHCl_2$ group is able to engage itself in an alternating rotation of the *o*-substituted aryl components, through an oscillating movement, so that the *o*-substituent of the turning rings turns past the H atom of the $CHCl_2$ group. While in *o,o*′-DDT (Figs. 11–13) the different positions of the molecular components cannot be converted into one another, this is possible with *o,o*′-DDD, although this conversion, naturally is easier with derivatives of the DDD series not substituted in the *o*-position.

H. α,β,β,β-TETRACHLORO- AND α,β,β-TRICHLORO-α,α-BIS-(4-CHLOROPHENYL)-ETHANES

The comparison of the α-Cl substitution products of *p,p*-DDT and *p,p*′-DDD is highly informative. With α,β,β,β-tetrachloro-α,α-bis-(4-chlorophenyl)-ethane (XII) (Fig. 17), the α-Cl atom obstructs the rotation of the CCl_3 group because of the strong repulsion which appears with the turning of the Cl atom of the CCl_3 group past the α-Cl atom. Consequently, it is natural also that a complete turning of the benzene rings is strongly hindered. However, if a Cl atom of the CCl_3 group is replaced by H, as is the case with α,β,β-trichloro-α,α-bis-(4-chlorophenyl)-ethane (XIII) (Fig. 18), the $CHCl_2$ group can swing back and forth, and therefore a complete turning of the benzene rings is possible. For comparison, Figure 19 shows a view of the model of *p,p*′-DDT corresponding with those of Figures 17 and 18.

I. THE STUART-BRIEGLEB MODELS OF DDT ANALOGUES

As emphasized, all of the preceding model studies were made with the models of Stuart, for these translate the spatial relationships most readily. However, we have also carried out corresponding studies with the newer Stuart-Briegleb models. This appeared essential to us, since the newer models are considerably altered, especially in the aromatic system. We established that the individual DDT analogues show the same differences in spatial build when constructed with the Stuart-Briegleb molecular models, but the larger carbon atom models seem to hinder the turning of the benzene

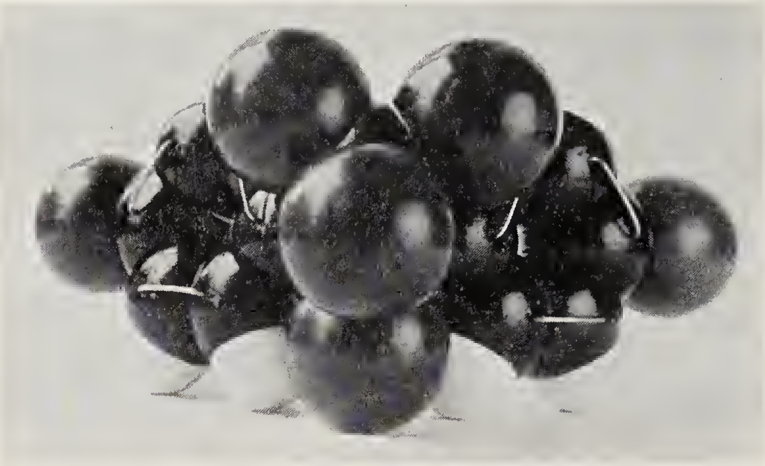


Figure 17

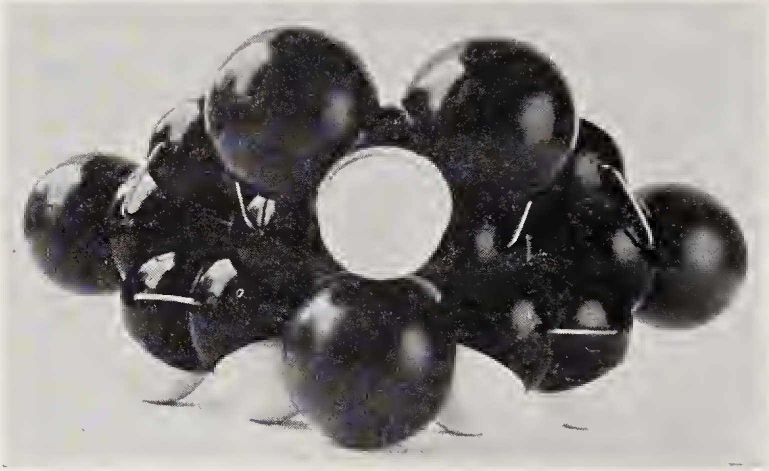


Figure 18

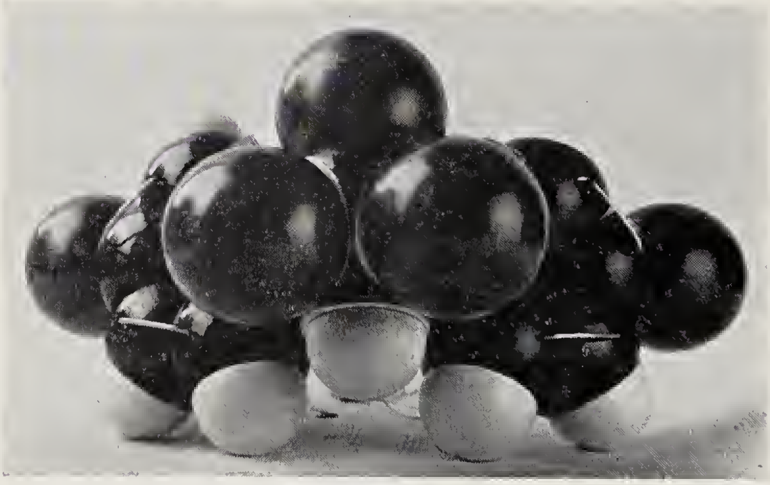


Figure 19

rings past the CCl_3 group in the case of the basic substance of the DDT analogues, β,β,β-trichloro-α,α-diphenylethane (II) (Figs. 20 and 21), more than those of the Stuart models. Nevertheless, the different positions of the molecular components can still be converted to one another.

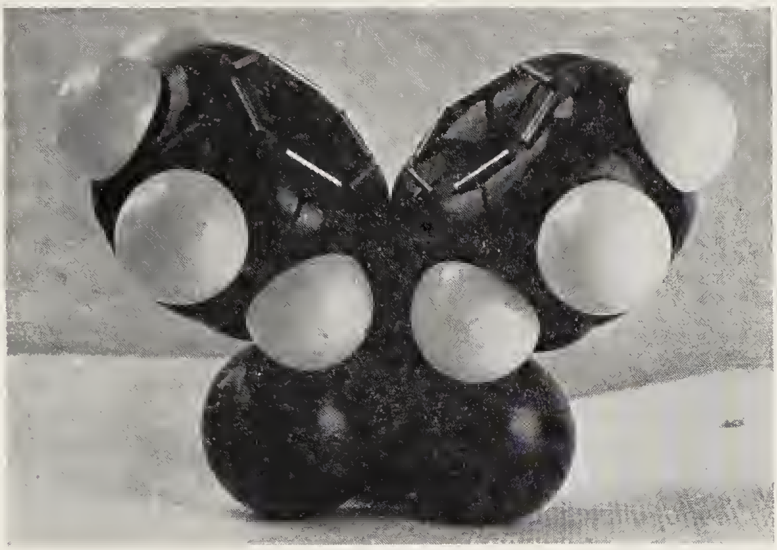


Figure 20

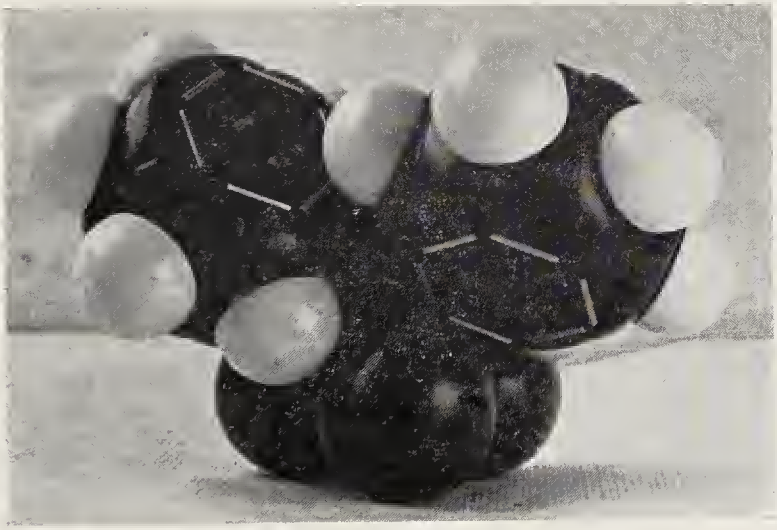


Figure 21

J. RESULTS OF THE MODEL INVESTIGATIONS

The model studies of the contactophores of DDT and its analogues demonstrate that the stereostructures of these compounds exhibit some peculiarities never or seldom apparent from the conventional structural formulas. The spatial structure of the molecule will be basically changed through the entrance of substituents in certain positions of the benzene rings so that the individual DDT analogues, in spite of "similar" constitution, can be strikingly different in their spatial structure, the position of the ring plane, their symmetrical properties, etc. These differences are especially large in relation to the degree of "free rotatability" of the benzene rings; for example, the rotatability is extensively or entirely curtailed in the *o*-substitution products and in all ethylene compounds.

Where there is "free rotatability," a *single* constellation series results with the observed DDT analogues, i.e., a *single* theoretically possible configuration. If not all the positions of the molecular components are interchangeable, for example, in DDT analogues with certain substituents in the *o,o'*-positions, additional constellation series result, i.e., more configurations are theoretically possible. Each of these configurations is either a series of constellations convertible from one to another, or identical with a constellation which is not convertible to any other.*

In the following section we present, as already mentioned, comparative observations of the spatial structure and the insecticidal activity of the DDT analogues. The experimental proof for the derived relationship between the contact insecticidal activity and the degree of rotatability of the molecular components is described in Section V.

IV. Configuration and Activity of DDT Analogues

In order to be able to relate the configuration of DDT analogues to one another and also to their activity data, we need an expression for the differences in spatial structure of these compounds. The most useful for this purpose seemed to us to be the degree of rotatability

* In order to be able to describe accurately the configuration of DDT molecules, we have quantitatively examined molecular models, in which we ascertained the constellations which belong to each configuration (the constellation series). For characterizing the constellations symbols have been employed instead of photographs. For further details see *Z. Naturforsch.*, *11b*, 38–43 (1956).

of the benzene rings and the CCl_3 (and the $CHCl_2$ and $CCl{=}CCl_2$) groups. We distinguished between:

1. Completely "free rotatability," designated as + +, if all the possible positions of the molecular components of the model are interchangeable with one another, i.e., one constellation series is theoretically possible. The number of theoretically possible configurations = 1.

2. Impaired "free rotatability," designated as −, if not all of the possible positions of the molecular components of the model are interchangeable with one another, i.e. several constellation series are theoretically possible. The number of theoretically possible configurations is >1.

3. Partial "free rotatability," designated as +, a transition between *1* and *2*.

For the comparative evaluation between configuration and activity, we have naturally considered only those DDT analogues in which, according to the criteria evaluated in Section II, insecticidal activity can be expected. For example, the DDT analogues β,β,β-trichloro-α,α-bis-(4-hydroxyphenyl)-ethane (XIV), m.p. 206°, and β,β,β-trichloro-α,α-bis-(4-phenylphenyl)-ethane (XV), m.p. 152°, are certainly comparable with p,p'-DDT in spatial structure (see the model of β,β,β-trichloro-α,α-bis-(4-X-phenyl)-ethane in Section III). In both cases, however, the contactophore basic substance, β,β,β-trichloro-α,α-diphenylethane (II), bears negative auxocontact substituents. The lipoid solubilities of XIV and XV are small, and their melting points are relatively high, so that for these reasons they are not considered further.

In the statement concerning activity we referred to the test values of the papers listed in Table I. Table VI presents data on the degree of rotatability of the molecular components and the activity of fifty DDT analogues. Activity is expressed as the speed of action to chosen test animals. The activity of *Melophagus ovinus* L. is expressed in terms of the time in hours required by 3 mg. of DDT compound, applied as a 10% dust, to kill 80–100% of a group of twenty-five test animals, over a maximum observation time of 10 hours, rather than in terms of "toxic values," as in Communications I and II of this Series (31). The vinegar fly, *Drosophila melanogaster* M., has been employed as another test animal. The evaluation of the DDT analogues was carried out in the petri dish film test. The activity to

Drosophila melanogaster M. is given as the time in minutes in which 1–5 mg. of DDT analogue, dissolved in acetone, caused the death of a group of twenty test animals, over a maximum observation time of 300 minutes (34).

Toxic values are listed in Table VII for five DDT and three DFDT isomers to the house fly, *Musca domestica* L., and the granary weevil, *Calandra granaria* L., using the bell-jar and petri dish–filter paper tests (34).

If we now compare the activity of the individual DDT analogues, from Table VI with their configuration, it will be observed that in all DDT analogues of the contactophores β,β,β-trichloro-α,α-diphenylethane (II), β,β-dichloro-α,α-diphenylethane (III), β,γ,γ-trichloro-α,α-diphenylpropylene (V), and α,β,β-trichloro-α,α-diphenylethane (IV), substituted in the 4- and the 4,4′-positions with positive auxocontacts, good to very good insecticidal activity is associated with complete "free rotatability" of the benzene rings. The shift of a substituent from the 4- to the 3-position (compounds 10 and 11) (compounds 15 and 16) did not essentially affect the contact insecticidal activity of the compound, and the "free rotatability" of the benzene rings was only slightly changed. As soon, however, as a substituent is introduced into the 2-position, the activity is greatly decreased; 2,2′-substituted compounds are in general completely inactive, and in this type of substitution the "free rotatability" of the benzene rings is abolished. For the well-known five DDT isomers and three DFDT isomers, the activity decreases in the order stated in Table VII: $p,p' > m,p' > o,p' > m,o' > o,o'$. Here, too, there is a complete correlation between activity and "free rotatability" of the benzene rings and CCl_3 group. It is very remarkable that the gradation of activity, not only in relation to the speed of action but also in regard to the effective threshold concentration, corresponds to the decreased degree of rotatability (Table VII).

Of the tetrasubstitution products only those substituted in the 3,3′, 4,4′-positions show contact insecticidal activity, while the DDT analogues with substitution in the 2,2′,5,5′-positions are completely inactive. This is because of the *o*-substitution which permits no "free rotatability" (Table VI).

With the DDT analogues derived from the contactophores β,β-dichloro-α,α-diphenylethane (II) (DDD series) and β,γ,γ-trichloro-α,α-diphenylpropylene (V) (propylene series), the activity

TABLE VI

Contact Insecticidal Activity and Configuration of Some DDT Analogues

	DDT analogue		Insecticidal activity		Configuration degree of free rotatability
No.	*x*	*x'*	*Melophagus ovinus* L., hr.	*Drosophila melanogaster* M., min.	
	β,β,β-Trichloro-α,α-bis-(*x*-*x'*-phenyl)-ethane				
1	4-H	4-H	6		++
2	4-CH_3	4-CH_3	3		++
3	2-CH_3	4-CH_3	>10[a]	300[a]	+
4	4-C_2H_5	4-C_2H_5	2.5		++
5	3-CH_3·4-CH_3	3-CH_3·4-CH_3	3		++
6	2-CH_3·4-CH_3	2-CH_3·4-CH_3	>10		−
7	2-CH_3·5-CH_3	2-CH_3·5-CH_3	>10		−
8	2-CH_3·4-CH_3.5-CH_3	2-CH_3·4-CH_3.5-CH_3	>10	>300[a]	−
9	2-C_2H_5·5-C_2H_5	2-C_2H_5·5-C_2H_5		>300	−
10	4-F	4-F	0.25	<10	++
11	3-F	4-F	0.25	<10	++
12	2-F	4-F	2.5	30	+
13	4-Cl	4-H	2		++
14	4-CH_3	4-Cl	2.5		++
15	4-Cl	4-Cl	0.5	40–50	++
16	3-Cl	4-Cl	2	40–50	++
17	2-Cl	4-Cl	6	130	+
18	2-Cl	3-Cl	6–9		+
19	2-Cl	2-Cl	>10	>300	−
20	4-Br	4-Br	4		++
21	2-Br	4-Br		300	+
22	4-CH_3·3-F	4-CH_3·3-F		55	++
23	2-CH_3·4-F	2-CH_3·4-F		>300	−
24	2-CH_3·5-F	2-CH_3·5-F		>300	−
25	4-CH_3·3-Cl	4-CH_3·3-Cl		270	++
26	2-CH_3·4-Cl	2-CH_3·4-Cl		>300	−
27	2-CH_3·5-Cl	2-CH_3·5-Cl		>300	−
28	4-CH_3·3-Br	4-CH_3·3-Br	9	280	++
29	2-CH_3·4-Br	2-CH_3·4-Br	>10	>300	−
30	2-CH_3·5-Br	2-CH_3·5-Br	>10	>300	−
31	4-CH_3O	4-CH_3O	2.5	>300	++
32	2-CH_3O	4-CH_3O	10	>300	+
33	4-C_2H_5O	4-C_2H_5O	4	>300	++

TABLE VI (*Continued*)

Contact Insecticidal Activity and Configuration of Some DDT Analogues

No.	DDT analogue		Insecticidal activity		Configuration degree of free rotatability
	x	x'	*Melophagus ovinus* L., hr.	*Drosophila melanogaster* M., min.	
	β,β-Dichloro-α,α-bis-(x,x'-phenyl)-ethane				
34	4-H	4-H	>10		++
35	4-CH_3	4-CH_3	3.5		++
36	2-CH_3	4-CH_3		300	+
37	4-F	4-F	1	30	++
38	2-F	4-F		150	+
39	4-Cl	4-Cl	2	45	++
40	2-Cl	4-Cl		200	+
41	4-Br	4-Br	6		++
	β,γ,γ-Trichloro-α,α-bis-(x-x'-phenyl)-propylene				
42	4-H	4-H	9		++
43	2-$CH_3\cdot$4-CH_3	2-$CH_3\cdot$4-CH_3	12		++
44	3-$CH_3\cdot$4-CH_3	3-$CH_3\cdot$4-CH_3	14		++
45	4-F	4-F	7	200	++
46	4-Cl	4-Cl	9	300	++
	α-Halogen Substitution Products of p,p'-DDT and p,p'-DDD α,β,β,β-Tetrachloro-α,α-bis-(x-x'-phenyl)-ethane				
47	4-Cl	4-Cl	>10	>300	–
48	4-F	4-F	>10	>300	–
	α,β,β-Trichloro-α-bromo-α,α-bis-(x-x'-phenyl)-ethane				
49	4-Cl	4-Cl	>10	>300	–
	α,β,β-Trichloro-α,α-bis-(x-x'-phenyl)-ethane				
50	4-Cl	4-Cl	4		++

[a] >10 hours as well as >300 minutes is equivalent to inactivity during the period of observation.

differences between the compounds with and without *o*-substituents are slight in all cases. This conforms with the finding that with these compounds, even in the presence of *o*-substituents, almost no

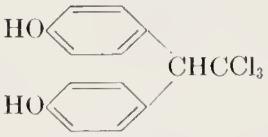
TABLE VII
Activity of Five DDT and Three DFDT Isomers to *Musca domestica* (Bell Jar Test) and *Calandra granaria* (Petri Dish–Filter Paper Test) (34)

Isomer	*Musca domestica* L. 50% mortality in 20 hr. from application of	*Calandra granaria* L. mortality from application (per dish) of		
		200 mg.	100 mg.	60 mg.
p,p′-DDT	16–20γ			88%
m,p′-DDT	16–24γ			93%
o,p′-DDT	800–1200γ		<50%	
o,m′-DDT	>1200γ	<50%		
o,o′-DDT	>1600γ	<50%		
p,p′-DFDT	12–24γ			100%
m,p′-DFDT	20–30γ			100%
o,p′-DFDT	800–1000γ		<50%	

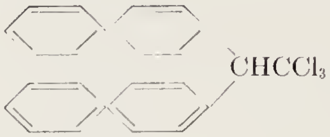
hindrance of the "free rotatability" of the benzene rings and the $CHCl_2$ and $CCl{=}CCl_2$ groups occurs (Table VI). If one passes, however, from the propylene to the ethylene series, inactivity is found in all cases. These facts are completely in accord with observations of the models. In the ethylene compounds of the DDT series the "free rotatability" of the benzene rings is encroached upon by the strong $C{=}CCl_2$ binding.

A comparison between the activities of α,β,β,β-tetrachloro-α,α-bis-(4-chlorophenyl)-ethane (XII) (compound 47, Table VI) and α,β,β-trichloro-α,α-bis-(4-chlorophenyl)-ethane (XIII) (compound 50) is especially interesting. While XII, which differs from DDT only through substitution of the α-H atom by Cl, has almost no insecticidal activity, XIII shows strong activity. The rule that compounds with the CCl_3 group are superior in toxicity to their homologues with the $CHCl_2$ group is up to now only broken in this case. The model observations also show, in this instance, a parallel between rotatability and insecticidal activity. While the α-Cl atom deprives XII of "free rotatability" of the CCl_3 group, and also of the benzene rings, such rotatability is possible with XIII because of the smaller volume of the $CHCl_2$ group.

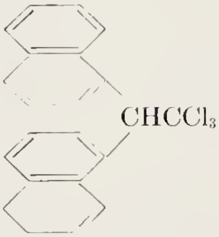
We thus prove in all cases a *correlation between insecticidal activity and the degree of rotatability of the molecular components of the DDT analogues.* Throughout the DDT series *ortho* substitution is unsuitable for insecticidal activity. This explains why such a great number of compounds which have been synthesized and tested are inactive, even though, according to the earlier concepts, they should be active. For example, the derivatives of β,β,β-trichloro-α,α-bis-(naphthyl)-ethane (XVI) are collectively inactive. In this case, the second ring acts as an *o*-substituent of especially large volume, whose rotatability is also completely abolished, while the basic substance (XVI) is not a contactophore. The corresponding is true of β,β-dichloro-α,α-diphenylethylene (XI).



(XIV)



(XV)



(XVI)

The question of whether the theory established here can be brought into harmony with the experimental results of other authors (Table I) can be answered in a positive way. We have endeavored to draw together all works in which toxicity values for DDT analogues are described and have compared these test values with the degree of free rotatability that is present. This extensive project was facilitated by the tabulation of a considerable portion of these toxicity values already made by the writer (1948); these are concerned with investigations on the following test organisms: *Calliphora vomitoria*, *Musca domestica*, and larvae of *Anopheles quadrimaculatus* and *Culex quinquefasciatus*. Not all of this tabulated information is repeated here because it concerns very many DDT analogues and has complete analogy to Tables VI and VII. A few other interesting cases have been gathered together in Table VIII. It is evident that the other investigators have found inactivity in all the fourfold substituted DDT analogues with substituents in the 2,2′-positions. Especially remarkable are compounds 10 and 11 of Table VIII: β,β,β-trichloro-

TABLE VIII
Contact Insecticidal Activity and Configuration of Some DDT Analogues

No.	DDT analogue x	DDT analogue x′	Insecticidal activity: *Culex quinquefasciatus* larvae [a]	Insecticidal activity: *Musca domestica* [b]	Configuration degree of rotatability
	β,β,β-Trichloro-α,α-bis-(x-x'-phenyl)-ethane				
1[c]	$4\text{-}CH_3O\cdot 3\text{-}CH_3$	$4\text{-}CH_3O\cdot 3\text{-}CH_3$	0.05–0.2		++
2	$4\text{-}CH_3O\cdot 2\text{-}CH_3$	$4\text{-}CH_3O\cdot 2\text{-}CH_3$	>4.0		−
3	$2\text{-}CH_3O\cdot 5\text{-}CH_3$	$2\text{-}CH_3O\cdot 5\text{-}CH_3$	>4.0		−
4	$2\text{-}CH_3O\cdot 5\text{-}CH_3O$	$2\text{-}CH_3O\cdot 5\text{-}CH_3O$	>4.0	2	−
5	$2\text{-}C_2H_5O\cdot 5\text{-}CH_3$	$2\text{-}C_2H_5O\cdot 5\text{-}CH_3$	>4.0		−
6[c]	$4\text{-}CH_3O\cdot 3\text{-}Cl$	$4\text{-}CH_3O\cdot 3\text{-}Cl$	0.05–0.2		++
7	$2\text{-}CH_3O\cdot 5\text{-}Cl$	$2\text{-}CH_3O\cdot 5\text{-}Cl$	>4.0	3	−
8	$2\text{-}(5)\text{-}CH_3\cdot 5\text{-}(2)\text{-}Cl$	$2\text{-}(5)\text{-}CH_3\cdot 5\text{-}(2)\text{-}Cl$	>4.0		−
9	$4\text{-}CH_3O\cdot 2\text{-}CH_3\cdot 6\text{-}CH_3$	$4\text{-}CH_3O\cdot 2\text{-}CH_3\cdot 6\text{-}CH_3$	>4.0		−
10	$4\text{-}CH_3O\cdot 3\text{-}Cl\cdot 5\text{-}Cl$	$4\text{-}CH_3O\cdot 3\text{-}Cl\cdot 5\text{-}Cl$		100	++
11	$2\text{-}OH\cdot 3\text{-}Cl\cdot 5\text{-}Cl$	$2\text{-}OH\cdot 3\text{-}Cl\cdot 5\text{-}Cl$		(100)	−
12[c]	4-Cl	4-Cl	0.01–0.05	100	++

[a] Concentration, in g. × 10^{-6}, for 50% mortality (22).
[b] Per cent mortality from application of 1% solution (10).
[c] Numbers 1, 6, and 12 were taken together for comparison.

α,α-bis-(4-methoxy-3,5-dichlorophenyl)-ethane and β,β,β-trichloro-α,α-bis-(2-hydroxy-3,5-dichlorophenyl)-ethane, for these more than fourfold substituted DDT analogues should possess insecticidal activity according to references in the literature. There is no objection to the activity of the first of these (compound 10, Table VIII), for the compound possesses free rotatability of the molecular components; but the conclusions of the considerations stated above contradict the stated insecticidal activity of the second (compound 11, Table VIII) toward *Musca*. This compound possesses two *o*-substituents and therefore has no free rotatability of the benzene rings and CCl_3 group comparable to *p,p′*-DDT. For this and other reasons, we carefully re-examined the information and found, in concurrence with the model observations, the complete inactivity of this DDT analogue.

Regarding another point, it was previously shown that the compounds formulated by C. Weygand as hexachloro-β,β-bis-(chlorophenyl)-propane (XVII), α,α,α-trifluoro-β,β-bis-(chlorophenyl)-propane (XVIII), β,β,β-trichloro-α,α,α-triphenylethane (XIX), and α,α,α-tri-(chlorophenyl)-α-trichloromethylmethane (XX) possess a completely different composition. Compounds "XVII" and "XVIII," recognized as active, cannot be considered as DDT analogues. If the constitution of "XVII–XX" had not been accurately determined previously, our considerations regarding the spatial structure of active DDT analogues would have given an impulse to check against the constitution as well as the test evaluation which was employed with these compounds. The insecticidal activity of "XVII" and "XVIII" could not be compatible with the hindrance of the "free rotatability" of the aromatic rings and the trihalomethyl group(s).

V. Experimental Investigations of the Degree of Rotatability of the Molecular Components of a Few DDT Analogues

In order that we may make an assertion regarding the degree of rotatability of the molecular component of DDT analogues—that is, in order to support the hypothesis derived from considerations of models and test results concerning the "correlation between contact insecticidal activity and degree of rotatability"—we have conducted dipole measurements on suitable DDT analogues.

In the earlier publications about dipole measurements of DDT analogues (15–17,20,50), the problem of rotatability of the molecular components was only touched upon. The compounds investigated permitted no definitive statements about the conversion of different theoretical constellations from one DDT analogue to another. The unsymmetrical substituted DDT analogues XXI and XXII make uncertain simple hypotheses which are indispensable for the calculation of their dipole moments. In the condensation products of the three isomers of xylene and chloral (XXIII–XXV) one comes to expect, partly on the strength of model observations, that the expected hindered rotatability of the components is hardly to be manifested in measured dipole moments since *o*- and *m*-xylene themselves possess only small dipole moments. With compound XXV no general statement can be made, as the moment of *p*-xylene is zero.

The condensation products of the halogenated toluenes with chloral appeared to us as suitable for these investigations, particularly those DDT analogues synthesized through condensation of the *o*-, *m*-, and *p*-isomers of fluoro-, chloro-, and bromotoluene with chloral, tabulated in Table IX. Our investigations of the structures of the synthesized condensation products of halogenated toluenes and chloral have shown that only the DDT analogues obtained from *o*-substituted toluenes possess *no* *o*-substitution (compounds 1, 4, and 7, Table IX). The dipole moments of the isomeric halogenated toluenes are considerable so that the demonstrated more or less large rotatability of the molecular components is manifested in the measured dipole moments. Because under definite assumption (41,42) the dipole moments of these DDT analogues (Table IX) can be calculated, a comparison of the measured values with the results of model observations is possible.

TABLE IX

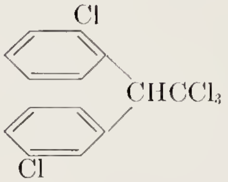
β,β,β-Trichloro-α,α-bis-(x,x'-phenyl)-ethanes

No.	x	x'	Melting point, °C.	Dipole moment, μ in D[a]	Insecticidal activity[b]
1	4-F	3-CH_3	58.5–59.5		+
2	4-F	2-CH_3	(b.p. 191/5 mm.)		−
3	5-F	2-CH_3	109		−
4	4-Cl	3-CH_3	78–80	1.88	+
5	4-Cl	2-CH_3	107.8–108	2.46	−
6	5-Cl	2-CH_3	158	3.13	−
7	4-Br	3-CH_3	108–109	1.85	+
8	4-Br	2-CH_3	100–101	2.38	−
9	5-Br	2-CH_3	182–183	3.22	−

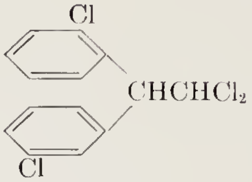
[a] Measured in benzene at 25°C. (41).

[b] Table VI.

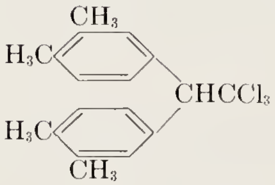
Concerning the statements which we are able to make about the constellation series (configurations) of the DDT analogues listed in Table IX, and the degree of rotatability of their molecular components, we have *measured* and calculated the dipole moments and compared the two sets of values. We proceed from the model observations employed in Section III. The β,β,β-trichloro-α,α-bis-(4-halogen-2-methylphenyl)-ethane (XXVI) and the β,β,β-trichloro-α,α,-bis-(5-halogen-2-methylphenyl)-ethane (XXVII) (compounds 2, 3, 5, 6, 8, and 9, Table IX)—in contrast to the β,β,β-trichloro-α,α-bis-(4-halogen-3-methylphenyl)-ethane (XXVIII) (compounds 1, 4, and 7, Table IX)—are compounds from which more than one non-convertible constellation series (configuration) can be described at the model in regard to hindered rotatability of the aryl nucleus. For these to be valid, the requirements in Section III.D must be fulfilled.


Cl
CHCCl3
Cl

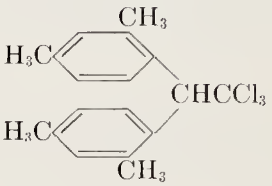
(XXI)


Cl
CHCHCl2
Cl

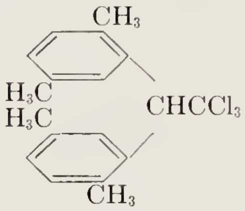
(XXII)


CH3
H3C
CHCCl3
H3C
CH3

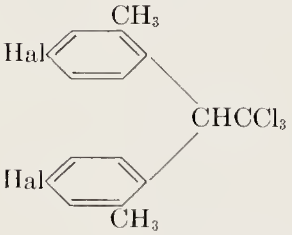
(XXIII)


CH3
H3C
CHCCl3
H3C
CH3

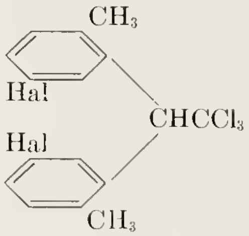
(XXIV)


CH3
H3C
H3C
CHCCl3
CH3

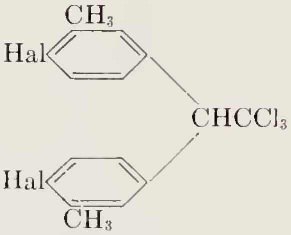
(XXV)


CH3
Hal
CHCCl3
Hal
CH3

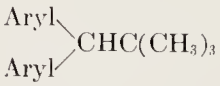
(XXVI)


CH3
Hal
CHCCl3
Hal
CH3

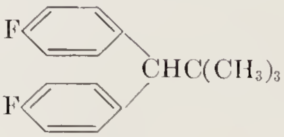
(XXVII)


CH3
Hal
CHCCl3
Hal
CH3

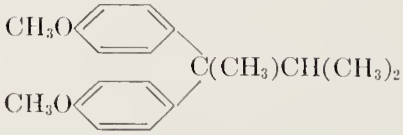
(XXVIII)


Aryl
CHC(CH3)3
Aryl

(XXIX)



(XXX)


CH3O
C(CH3)CH(CH3)2
CH3O

(XXXI)

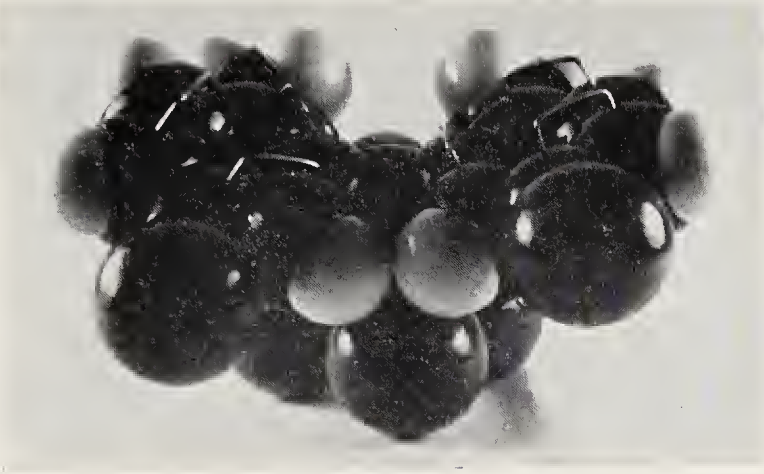


Figure 22

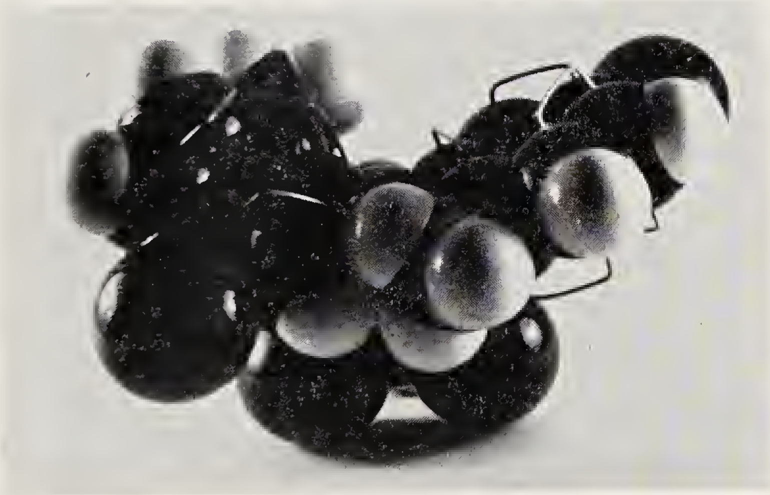


Figure 23

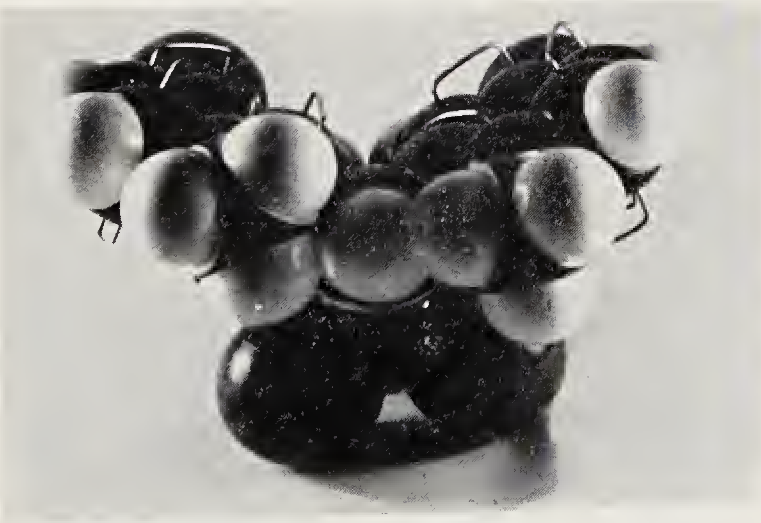


Figure 24

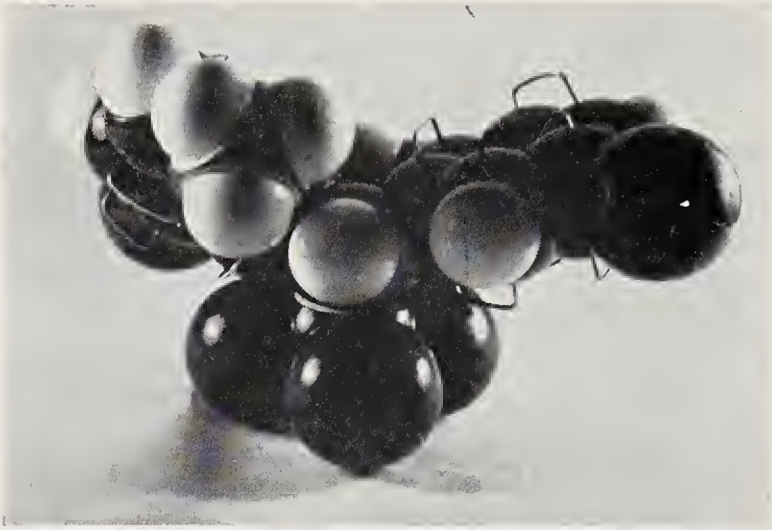


Figure 25

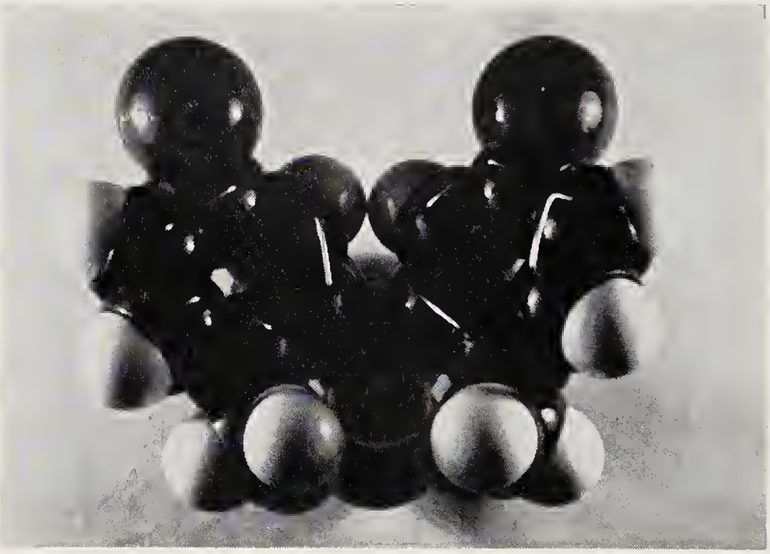


Figure 26

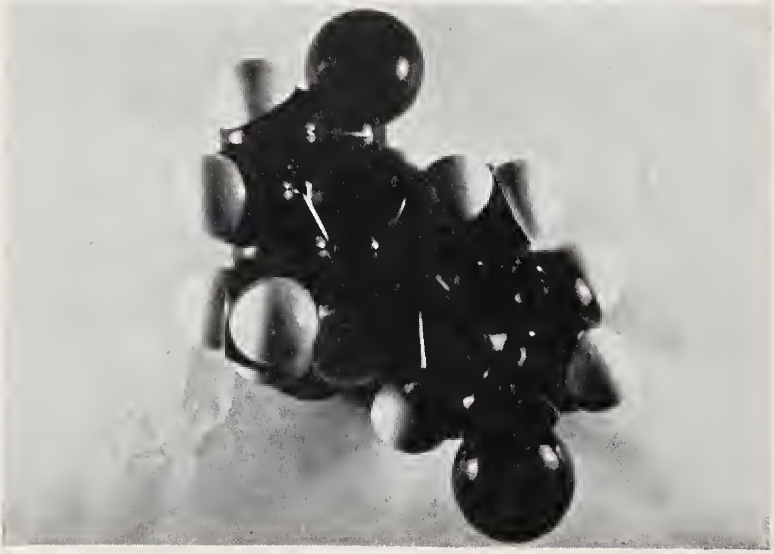


Figure 27

Two constellations of β,β,β-trichloro-α,α-bis-(5-chloro-2-methylphenyl)-ethane are shown in Figures 22–27. Figure 22 corresponds with Figure 2, both Cl atoms in the 5-position in front of, and both methyl groups in the 2-position behind, the plane of the picture. In Figure 23 one Cl atom is in front and one Cl atom is behind. Figure 24 shows once again the constellation of β,β,β-trichloro-α,α-bis-(5-chloro-2-methylphenyl)-ethane previously shown in Figure 22, but with the α-H atom toward the observer. The corresponding is true with Figures 25 and 23. In Figures 26 and 27 the constellations shown in Figures 24 and 25 are photographed from above (CCl_3 group not visible). The illustrations of the corresponding constellations of β,β,β-trichloro-α,α-bis-(4-chloro-2-methylphenyl)-ethane are omitted, for they show nothing new.

We have calculated the theoretical dipole moments of the symmetrical constellations of β,β,β-trichloro-α,α-bis-(5-halogen-2-methylphenyl)-ethane and β,β,β-trichloro-α,α-bis-(4-halogen-2-methylphenyl)-ethane, using values for the valence angles, α ond β, of the α-C atom (Fig. 3) determined from simpler DDT analogues (41):

β,β,β-Trichloro-α,α-bis-(5-chloro-2-methylphenyl)-ethane
  Constellation shown in Figure 22: 3.15 D ($\alpha = \beta = 114°$)
  Constellation shown in Figure 23: 1.40 D ($\alpha = \beta = 114°$)
β,β,β-Trichloro-α,α-bis-(4-chloro-2-methylphenyl)-ethane
  Constellation corresponding to Figure 22: 2.34 D ($\alpha = \beta = 110°$)
  Constellation corresponding to Figure 23: 1.76 D ($\alpha = \beta = 110°$)
β,β,β-Trichloro-α,α-bis-(5-bromo-2-methylphenyl)-ethane
  Constellation corresponding to Figure 22: 3.22 D ($\alpha = \beta = 114°$)
  Constellation corresponding to Figure 23: 1.15 D ($\alpha = \beta = 114°$)
β,β,β-Trichloro-α,α-bis-(4-bromo-2-methylphenyl)-ethane
  Constellation corresponding to Figure 22: 2.34 D ($\alpha = \beta = 110°$)
  Constellation corresponding to Figure 23: 1.74 D ($\alpha = \beta = 110°$)

Measured dipole moments were 3.13 D (halogen = Cl) and 3.22 D (halogen = Br) for β,β,β-trichloro-α,α-bis-(5-halogen-2-methylphenyl)-ethane and 2.46 D (halogen = Cl) and 2.38 D (halogen = Br) for β,β,β-trichloro-α,α-bis-(4-halogen-2-methylphenyl)-ethane. A comparison of the measured and calculated dipole moments shows immediately that the constellations of these compounds conform to Figure 22. Also the comparison of the calculated dipole moments for free rotatability of:

β,β,β-Trichloro-α,α-bis-(5-chloro-2-methylphenyl)-ethane
2.66 D ($\alpha = \beta = 114°$)
β,β,β-Trichloro-α,α-bis-(4-chloro-2-methylphenyl)-ethane
1.39 D ($\alpha = \beta = 114°$); 1.81 D ($\alpha = \beta = 110°$)
β,β,β-Trichloro-α,α-bis-(5-bromo-2-methylphenyl)-ethane
2.66 D ($\alpha = \beta = 114°$)
β,β,β-Trichloro-α,α-bis-(4-bromo-2-methylphenyl)-ethane
1.39 D ($\alpha = \beta = 114°$); 1.80 D ($\alpha = \beta = 110°$)

confirms this conclusion. The difference between the measured and calculated dipole moments is large enough to exclude "free rotatability" on the one hand and to provide the decision in favor of the indicated constellation on the other.

While measurement and calculation of the dipole moment excludes "free rotatability" and permits classification of β,β,β-trichloro-α,α-bis-(4-halogen-2-methylphenyl)-ethane (XXVI) and β,β,β-trichloro-α,α-bis-(5-halogen-2-methylphenyl)-ethane (XXVII), a similar procedure does not lead to a definite constellation for β,β,β-trichloro-α,α-bis-(4-halogen-3-methylphenyl)-ethane (XXVIII), as was to be expected from the model observations (cf. Sections III.B and III.C). Using the formula given elsewhere (42), one comes, however, to the conclusion that the measured 1.85 D of β,β,β-trichloro-α,α-bis-(4-bromo-3-methylphenyl)-ethane coincides very well with the calculated dipole moment for the oscillation of the aryl portion of $\tau = \pm 140°$. Since, in the calculation, an oscillation of $\tau = \pm 180°$ signifies free rotatability, the measurement also confirms the model observations in the case of β,β,β-trichloro-α,α-bis-(4-bromo-3-methylphenyl)-ethane. The corresponding situation is true for β,β,β-trichloro-α,α-bis-(4-chloro-3-methylphenyl)-ethane (41).

The example given here of the investigation of the condensation products of the isomeric chloro-, bromo-, and fluorotoluenes with chloral therefore can be considered as support of the derived "correlation between contact insecticidal activity and degree of rotatability" (Section IV). Of the investigated condensation products only β,β,β-trichloro-α,α-bis-(4-halogen-3-methylphenyl)-ethane, in which the degree of rotatability is very large, shows insecticidal activity (Table IX).

The fact that the three condensation products of corresponding structure for chloral and *o*-, *m*-, and *p*-iodotoluene possess essentially no insecticidal activity (37) is in complete agreement with the fore going conclusion. The introduction of iodine increases the molecular weight of the DDT analogues above the admissible limit; hence, the isomeric β,β,β-trichloro-α,α,-bis-(iodomethylphenyl)-ethanes are eliminated at the outset from such consideration.

It is also possible to refer to the configuration of DDT analogues as a *further criterion* for the estimation of their contact insecticidal activity and from this to prove before by the synthesis of such materials, whether or not they possess insecticidal activity. In the search for new contactophores, a great deal of work could have been avoided if this new criterion had been made known earlier. The hitherto used criteria afforded no indication as to which condensation products could produce contactophores, for the only criteria known to us for the synthesis of DDT analogues were molecular weight and auxocontacts. By application of the criterion of configuration the number of DDT analogues to be assayed for insecticidal activity could have been concentrated markedly, and entire groups of compounds, for example, the naphthyl derivatives of DDT, mentioned above, could have been excluded at the outset.

Naturally it was valuable for the considerations employed here that such extensive research concerning the insecticidal properties of DDT analogues was carried out previously so that henceforth much of it relating to the essential goal of earlier research—the discovery of new active analogues of DDT—could be changed from its negative direction to become of positive value. Consideration of the *configuration* of the molecule provides an essentially better control over the dimensions of modification than the arrangement of the customary *structural* formula. Upon the basis of this sort of model observation, the modifications of the structure of DDT analogues which can be undertaken without fundamentally altering the *configuration*, and therefore the probable correlation with the activity ratios, can be judged.

Finally, it appears essential to comment upon the published work of Rogers and coworkers (44) concerning "Structure and Toxicity of DDT-Insecticides" (Table II) which, proceeding from the hypothesis of the so-called "trihedralization of DDT-analogues," has not reached conclusions which are possible on the basis of subsequent model observations and from the criteria for judging insecticidal activity of DDT analogues given in Section II.

Rogers and coworkers do not always clearly distinguish in their work between configuration and structure, which has been shown in the foregoing explanation of the problem to be absolutely essential. They have conducted their investigation not on the basic DDT substances but on methyl analogues (XXIX), which collectively are very weakly active, and also by means of test methods which allow only relatively uncertain conclusions. Moreover, to establish their interpretation, these authors are compelled to draw upon supplementary hypotheses (steroid theory). Their statements are made still more dubious by the fact that the compounds they tested, with two exceptions, could not be crystallized or their structures proved. It is characteristic that one of the two crystalline compounds (XXX), the activity of which would be very important for Rogers' theory, is completely inactive. In the publication of Rogers and coworkers, no related model observations are to be seen. Most important, they have not recognized the differences in spatial structure of the individually evaluated compounds; rather, they have sought to relate the activity of all investigated DDT analogues to a common denominator, namely, their "trihedralized configuration." They cannot, to be sure, transfer their observations to the essential DDT substances.

Primarily, however, the statements of Rogers and coworkers are disposed of by the fact in question that we have reached a completely opposite conclusion, through observation of models, with regard to the rotatability of the molecular components (aromatic rings or the aliphatic remainder) about the central carbon atom. In DDT substances which are not substituted in the *o*-position (Section III), the molecular components are not independent of one another but are capable of rotating with one another. If the aryl components are substituted in the *o*-position, as in the naphthyl rings, or if the C_α–C_β bond is a double bond (ethylene linkage), this rotatability is hindered. The corresponding is true of derivative XXIX, investigated by Rogers and coworkers. According to these investigators, the aryl components should occupy a nearly planar position, and their rotation should be hindered at the central carbon atom by the greater volume of the substituents; with *p,p'*-DDT itself only a *p*-chlorophenyl or the trichloromethyl group should be rotatable; and in the ethylene compounds of the DDT series the aryl groups should be freely rotatable. These statements contradict the model observations described in Section III. Also the assumed interpretation by Rogers and coworkers of the work of Wild (50) concerning the dipole moments of a few DDT analogues cannot prove true, for the conclusions from these measurements provide the best verification of our model observations and investigations and also directly contradict the assumed favored formation of the almost planar (trihedralized) position of the aryl components. Even modelwise, the nearly coplanar position of the aryl components, assumed by these authors is not possible without excessive distortion of the valence angle phenyl–C–phenyl (cf. Section V).

The trihedralization principle of Rogers and coworkers does not explain the differences in activity of the individual isomer groups of DDT and DFDT, for the substitution in *p*-, *m*-, and *o*-positions is not influenced by the trihedralization effect. Even the steroid hypothesis referred to by them does not clarify these differences: that *m,p'*-DDT possesses nearly the same activity as *p,p'*-DDT (compounds 16 and 15, Table VI), while the distances of the aromatically bound Cl atoms, which must be determinative for the binding with the steroid, are different in both isomers. Even the comparisons of activity which the authors make between their described compounds and the halogenated DDT analogues are inadmissible. For example, the inactivity of α,α-dianisyl-α,β-dimethylpropane (XXXI) is placed in parallel with the inactivity of α,β,β,β-tetrachloro-α,α-bis-(4-chlorophenyl)-ethane (XII). A comparison would, however, be admissible only with α,β,β-trichloro-α,α-bis-(4-chlorophenyl)-ethane (XIII), which has been demonstrated to be active (compound 50, Table VI).

A further decided objection to the "trihedralization principle" is derived from the inactivity of β,β-dimethyl-α,α-bis-(4-fluorophenyl)-propane (XXX), inasmuch as the corresponding Cl analogue, β,β,β-trichloro-α,α-bis-(4-fluorophenyl)-ethane (VI) (compound 10, Table VI) has the highest activity of all the DDT analogues. Rogers believes that the inactivity of XXX can be explained by the fact that the distance between the aromatically bound F atoms is less than the "critical length," but this distance must be the same in the highly active VI. The inactivity of XXX is, in the opinion of the writer, due to the fact that the basic molecule (XXIX) cannot be considered a contactophore and therefore all derivatives of this basic substance should be inactive or at the most weakly active. The activity shown by the "1,1-di-*p*-chloro-" and 1,1-di-*p*-"bromophenyl-2,2-dimethylpropane" compounds is perhaps related to this, in that these, produced through direct halogenation of the basic substance (XXIX), actually contain halogen in the aliphatic instead of the aromatic part of the molecule (and therewith are converted into compounds which are more closely related to the DDT analogues than XXIX). It is significant that the three analogues of XXIX, α,α-bis-(4-chlorophenyl)-, α,α-bis-(4-methylphenyl)-, and α,α-bis-(4-methoxyphenyl)-β,β-dimethylpropanes, which were prepared by Skerrett and Woodcock in 1950 (46,47) by an unequivocable method of synthesis, were completely inactive in test investigations of Skerrett with *Calandra granaria*, yet it is upon these compounds that Rogers and coworkers support their hypothesis. For these and other reasons it appears unreasonable to apply the new criterion "configuration," in the sense of free rotatability of the molecular components, to the class of compounds (XXIX) of Rogers and coworkers.

The interpretations developed by the above-named authors concerning the shape of the DDT molecule ("trihedralization") and of the relationship between "trihedralization" and insecticidal activity also cannot stand thorough re-examination. Continued investigation of structure and insecticidal activity of DDT analogues on the basis of this hypothesis promise little success.

VI. Summary

A comparison between configuration, i.e., the spatial structure, of DDT analogues and their insecticidal activity leads to more extensive experimental disclosures than those resulting from the employment of the customary planar structural formulas. This is true, however, only if model observations, which establish the differences in spatial structures of the DDT analogues, are carried out and related to the activity data, and if the criteria used for the evaluation of the insecticidal activity of the DDT analogues are taken into account.

The derived correlation between the contact insecticidal activity and the degree of rotatability of the molecular components of the DDT analogues is supported through the measurement of dipole moments of suitable DDT analogues. The configuration of the DDT analogues can, therefore, be considered as a further criterion for judgment of their activity.

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THE SPREAD OF INSECTICIDE RESISTANCE IN PEST SPECIES

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CONTENTS

I. Introduction 351
II. General Aspects of Insecticide Resistance 352
III. Resistance in Insect Pest Species 356
References 397

I. Introduction

Barely a half century after it was first observed by Melander (319), insecticide resistance is now present and proven in populations of some 60 species of arthropods. More than half of this number are DDT-resistant, and more than one-third are resistant to the other chlorinated hydrocarbons such as BHC, dieldrin, chlordane, toxaphene, or endrin; at least 10 are resistant to organophosphorus compounds. Diptera of medical importance constitute two-fifths of the species, and an equal proportion are agricultural pests in the orders Hemiptera, Lepidoptera, or Coleoptera.

The resistance problem has been recently reviewed on physiological lines by Metcalf (328), on genetical by Crow (109), and on toxicological by Hoskins and Gordon (219). The physiology of resistance has been covered by Chadwick (90–92,94), and its biochemistry by Kearns (236) and Winteringham (510); detoxification has been reviewed by J. N. Smith (454) and discussed by Winteringham and Barnes (511). The genetics of resistance has also been reviewed by Milani (332,336), and the genetics of resistance in mosquitoes by Kitzmiller (255). Earlier work has been covered by Babers (15) and by Babers and Pratt (17), and more recent by Wiesmann (499), Grayson and Cochran (179), and Hoffmann (215). Resistance in medical entomology has been reviewed by Hess (208,209), Simmons (447), Busvine (75,77,78,80), and Brown (60a); resistance of mosquitoes by King (253) and Brown (59); and dieldrin resistance by

Ascher (12). Resistance in agricultural insects has recently been treated by Babers (16) and (Wiesmann (499). Reviews are now appearing in many different countries, e.g., France (269,270), Germany (298), Austria (232), Turkey (24), and Spain (164).

II. General Aspects of Insecticide Resistance

Since the production and spread of inheritable resistance are essentially a genetical problem, the chromosome numbers of the various species as compiled by Makino (307) and the formal genetics of the house fly as synthesized preliminarily by Milani (334) are of especial interest. That resistant strains were produced from normal populations by a process of Darwinian selection was first explained to economic entomologists in 1941 by H. S. Smith (453), and the subsequent years have confirmed this view on the basis of Mendelian genetics (109).

Insects do not acquire tolerance during their lifetime. Young *Bombyx* larvae fed small quantities of sodium arsenate did not become thereby less susceptible at maturity (88). *Galleria* larvae that survived doses of DDT, nicotine, or pyrethrum were made the more susceptible to doses given 1 week later (31). Adult house flies exposed to daily sublethal doses of diazinon, dieldrin, or DDT became progressively more susceptible to them (183), and pre-exposure of either normal or resistant strains increased their susceptibility to DDT (216). It has been found with *Pediculus* (101), as with *Musca* (198), that resistance increased in successive generations only if the levels of DDT applied were sufficient to cause some degree of mortality. There is no acquired tolerance, assuefaction, or mithridatism afforded by completely sublethal levels. *Drosophila* larvae have been reared for 50 generations in a sublethal concentration of DDT without any decrease in the DDT susceptibility (297).

Insecticides evidently do not induce mutations for resistance; indeed, they do not increase the normal mutation rate at all. No more X-chromosomal lethal mutations were induced by exposing adults or larvae of *Drosophila* to DDT (297) or to γ-BHC (398). An inheritable mutant form with outspread wings has appeared in *Musca* exposed to technical BHC in southern Brazil (421), but it has nothing to do with BHC resistance. It appeared possible that the normal mutation rate occurring during the period of use of the insecticide might originate resistance in some cases (342); but it has been found that years of selection cannot produce resistance in any isogenic strain of *Drosophila* if it lacks the genes for resistance in the first place (109). The conclusion is therefore that resistance arises not from postadaptation but from preadaptation. Indeed, DDT resistance of *Cimex hemipterus* on Taiwan probably derived from the 0.5% of resistant individuals present in unsprayed areas (95); and dieldrin resistance of *Anopheles gambiae* in northern Nigeria was characterized by a frequency of 0.4–6% heterozygotes for the resistant gene in villages not yet sprayed, and a frequency of 90% homozygotes in areas sprayed with dieldrin or BHC (10).

Resistance is developed faster when the selection pressure is high than when only a small proportion is culled in each generation (385); this has been established in *Musca* (401) and *Drosophila* (327). Intense pressure at the LD_{90} or above may, however, be no more effective than the LD_{50} level where the surviving population is numerically small (109,248). Resistance was accelerated when larval pressure was added to adult pressure on *Musca* (65), presumably because more intense selection and more individuals were thereby involved. Although three instances of resistance in *Anopheles* have been associated with larviciding, three have not (66).

The resistance thus developed is inherited when selection is discontinued; cyanide-resistant *Aonidiella* has maintained its level for 10 years (523). But DDT-resistant strains of *Musca* eventually reverted toward susceptibility, except in three instances only (65,114, 272). The reason for this reversion is obscure, since resistant strains characteristically have quite normal bionomics (328), instances of associated lethal genes (25) or low fecundity (306) being rare, and generally due to inbreeding (272). However, if the strain is nonhomogeneous for any genes which could affect resistance, some degree of variation among individuals will result; there is evidence that the more susceptible individuals develop faster, and thus could increase their proportion in the population because of their greater biotic potential (305).

Reversion under field conditions is, of course, mainly due to genic dilution with surrounding untreated populations. House fly resistance has decreased in California since 1952 when chlorinated hydrocarbons were discontinued (328), but a reverted strain will quickly regain resistance on the return of insecticide pressure. Larvæ of *Musca d.*

vicina in Egypt, which had lost their BHC resistance in a season of freedom, regained it in 7 weeks of exposure (387). Experimental respraying of Danish house flies restored in 4 weeks the DDT resistance that they had taken 5 years to lose (496).

Resistance to the modern organic insecticides, at least in house flies and mosquitoes, is evidently of three main types: (*1*) to DDT and its analogs, (*2*) to polychlorinated aromatics (BHC, dieldrin, chlordane, etc.), and (*3*) to organophosphorus compounds. Resistance of type *1* almost certainly derives from an ability to detoxify the DDT or analog by dehydrochlorinating it, but little is known of the physiological mechanisms involved in types *2* and *3*. In *Drosophila*, however, DDT tolerance also involves a tolerance of BHC (478) and other chlorinated hydrocarbons (497).

The strong and specific type *1* resistance in *Musca domestica* is evidently due to an allele of a single gene (197,237,271,306,333). The weaker tolerance in *Drosophila melanogaster*, which does not involve dehydrochlorination (109), has been found to be polygenic in origin (46,108,109,248); but in two Japanese strains one gene is paramount (269,478). Type *2* resistance has been found to be monofactorial in dieldrin-resistant *Anopheles gambiae*, but polygenic in BHC-resistant *Musca* (25,82,237) and chlordane-resistant *Blattella* (230).

The apparent resistance of the Colorado strain of *Carpocapsa pomonella* to arsenic is due to the fact that it had developed an increased size and toughness, which allow it to withstand desiccation better; it is no more resistant to arsenic than normal (222). This may be regarded as "vigor tolerance," a term recently introduced by Hoskins and Gordon (219) to cover cases where extra vigor imparts greater insecticide tolerance without involving any specific defense mechanism, and to explain the mild cross-resistance between the specific types. The original Orlando strain in which DDT tolerance was induced (285) was also tolerant of chlordane, toxaphene, pyrethrins, and Thanite (251,508); this was considered an example of vigor tolerance (328), and indeed the flies had become darker and more robust in appearance (251).

With the exception of the sex-linked gene for HCN resistance in *Aonidiella*, the genes for resistance are characteristically autosomal. However, maternal effects on the egg cytoplasm of the offspring are an element in the DDT resistance of *Blattella* (99,327) and in the slightly increased DDT tolerance of *Anopheles quadrimaculatus* (136).

The suggestion that DDT resistance in *Musca* derives from cytoplasmic granules produced under the influence of nuclear genes (235) is negated by the F_1 of reciprocal crosses being identical (327). The speed with which resistance developed under low selection pressures from dieldrin or γ-BHC at Basle led to the suggestion that it did not derive from gene selection alone (459).

Control failures have been observed to develop with 25 other species in addition to the 60 mentioned, but their insecticide resistance has not been confirmed owing to the lack of some set test to compare them to the normal population. Standardized tests are the most satisfactory, whereby mortalities are obtained at different doses of concentrations; from these the LD_{50} and LC_{50} figures are derived, and the degree of resistance may be gauged from their ratio of increase. When few insects are available for test, the speed to knockdown (KD_{50}) at a single concentration has been similarly utilized. House flies have been usually tested by topical application of the insecticide in solution; the use of mineral oil as a solvent avoids the exaggerated resistance ratios involved with acetone (27), which enhances the effect of low doses (26) and minimizes that of large amounts. The differential between strains is smaller the closer to the site of action that DDT is applied (265). Mosquitoes and *Drosophila* have usually been tested on residual deposits; here again long exposures to light deposits give exaggerated resistance ratios (79,108). The impregnation of oil solutions on paper has provided a test surface now widely used in medical entomology (83). Laboratory tests for agricultural insects have involved topical application for *Anthonomus* (432) and coated vials for *Lygus* (3).

Multivoltine insects are evidently more insecticide-tolerant in the autumn, as found for adult *Musca* (162,282), adult *Anopheles* (8,525), *Aedes* larvae (243), and presumably *Anthonomus* (7). The susceptibility levels change with age, instar, and state of nutrition, the literature on which has been reviewed by Potter (404). Females are characteristically more tolerant than males, being heavier. Individual insects may vary in tolerance from day to day and not maintain a static position in the population (30); this dynamic variation between days may be found to exceed the variation among individuals (45).

III. Resistance in Insect Pest Species

The species that are discussed below in taxonomic order do not constitute a list of resistant forms. They include 7 in which resistance is a laboratory production, 13 which are remarkable for the lack of induced resistance, and 7 in which claimed resistance is invalid. Moreover, resistance is an arbitrary attribute, implying that the population has changed so as to survive normal rates of insecticide application. An increasing number of species must be pressing toward this threshold, so that any review is out of date on publication.

Blattella germanica (German cockroach). DDT resistance was first observed in the field in the United States in 1952 (301) but in general has not developed in vector control programs (184,190). Laboratory selection for 12 generations has increased the LD_{50} of females 200 times and of males 20 times, and the insects were smaller and produced less offspring. The cross-resistance ratio to BHC was only 1.4–2.6 times (175,176). Hybrids between resistant females and normal males were intermediate in resistance, but hybrids between normal females and resistant males were scarcely resistant, although both F_2 generations were identical. The results suggest either that inheritance is partially cytoplasmic (99) or that maternal effects are involved, the egg cytoplasm being influenced by the maternal chromosomes before fertilization (327).

Chlordane resistance was first observed in 1951 at Corpus Christi. Texas (32), after four years of chlordane applications, the LD_{50} being increased 100 times (206). By 1955 the resistance was general in the Gulf Coast states, being about 20 times the normal in San Antonio, Jackson, and Phoenix (242), and in the southeastern states at least 20 populations were 10–25 times resistant (129). In 1956 resistance became prevalent in the northeastern states and the Chicago area (458). In 1958 it was reported from Alameda and the San Francisco Bay area of California (217a).

Cross resistance of the Corpus Christi strain (85,144,178,206) extends to dieldrin (70 times), in less degree to γ-BHC (10 times), and somewhat to DDT (5 times). This DDT tolerance is associated with a slightly greater production of DDE which is, however, an unimportant metabolite (20). Individuals of this strain were smaller and produced less offspring than normal (178). Hybrids with the normal strain were intermediate in resistance, reciprocal crosses being identical (179). Offspring of single-pair matings showed great variation

(230). It is therefore concluded that inheritance of chlordane resistance is autosomal and polygenic.

Pyrethrin resistance had also developed by 1956 in Texas and Alabama (241).

Blatta orientalis (Oriental cockroach). Specimens collected from treated premises in Rome showed less mortality to DDT in tests than those collected from untreated (420,447). This is not a case of developed DDT resistance, since it involved only survivors of the same generation that experienced the previous treatment.

Periplaneta americana (American cockroach). This species is more DDT-susceptible than *Blattella* and has not developed any DDT resistance in fector control programs (161). It converts DDT mainly to water-soluble conjugates of diphenylmethane derivatives, only 10% of it being excreted as DDE (426,484).

Pediculus humanus capitis (head louse). Head lice were quite normally susceptible to DDT in Korea, even on people infested with DDT-resistant body lice (225).

Pediculus humanus corporis (body louse). DDT resistance was first reported from Koje-do, Korea, and from Tokyo, Japan, in 1951, six years after the first use of the insecticide, only 50% mortality being shown to dust treatments that formerly killed 100% (28,225). Similar resistance developed in Palestine (209) and lower Egypt in 1952 (226). Cross resistance extended to methoxychlor (131), DANP (73), and Lauseto-Neu (100), but not to γ-BHC, dieldrin, toxaphene, or pyrethrins. By 1955 DDT resistance was present in Hong Kong, Iran, Turkey, Sudan, Ethiopia, coastal French West Africa, Union of South Africa, Peru, and Chile (515), and widespread in Japan (515, 517). Although European populations remained characteristically susceptible (264,292), infestations on some 5% of the individual hosts at Marseilles were decidedly DDT-resistant (366). The resistance of the Korean strain completely disappeared in 8 generations' relief from DDT (132); but was increased still further in 15 generations of pressure. With DDT pressure, the normal Orlando strain increased its resistance 40 times in 25 generations (132), and a Tokyo strain increased 60 times in 3 generations (516). Selections of a Sapporo strain with technical DDT increased its resistance to p,p'-DDT but not o,p'-DDT (518). Neither resistant lice nor normal lice absorb DDT through the cuticle. But resistant lice convert DDT *in vivo* not to DDE but to a water-soluble Schechter-Haller metabolite, and tissue extracts perform this *in vitro* if activated by glutathione (236, 389).

BHC resistance was discovered in two samples collected at Marseilles (366) and was present in most samples from Japan in 1955 (515). It developed in two villages and one town of northeastern Iran in 1956 (300a).

Scirtothrips citri (citrus thrips). Resistance to tartar emetic was noted in certain orchards in the San Fernando Valley, California, two years after its introduction in bait sprays in 1939. It was so strong that 4 times the usual dosage was inadequate, and 75% survival was shown to deposits that killed 99% of normal thrips (53). The resistance was not due to avoidance of this stomach poison and was stable for 7 generations of relaxed pressure (304). Resistance has continued to spread after cessation of the insecticide (328). In 1945 certain orchards in western Transvaal showed control failures that suggested this resistance (448). Evidence of tartar emetic resistance in the gladiolus thrips, *Taeniothrips simplex*, had appeared in California by 1943 (399). DDT resistance in the onion thrips, *T. tabaci*, has been reported in 1957 for a small area in southern Texas (153a). DDT resistance in the coffee thrips, *Diarthrothrips coffeae*, developed in 1956 at Oldeani, Tanganyika (470a).

Oncopeltus fasciatus (large milkweed bug). DDT resistance of 4 times the normal and toxaphene resistance 1.8 times the normal have been developed by 17 generations of laboratory pressure (177). *Oncopeltus* is normally fairly tolerant of DDT and detoxifies much of it to DDE (141). Nicotine tolerance has also been developed in the laboratory (481).

Lygus hesperus. DDT resistance of this plant bug appeared in 1952 and 1953 in the Yakima Valley, Washington, when mid-season DDT dusts on alfalfa failed to provide the control they had given since 1947 (323). Similar events occurred in the San Joaquin Valley, California, and tests in Kern County in 1953 showed the resistance to be 3–10 times the normal in late summer, but only 1–2 times in the spring. The greater LD_{50} in the later-season populations was found to be principally due to the elimination of the susceptible phenotypes without a shift in the dosage-mortality curve (4). DDT resistance also appeared in the Salt River Valley, Arizona, in 1953 and 1954, when DDT dusts on beets failed to control the young nymphs, which subsequently hatched, although they had done so since 1946 (210).

A slight increase in toxaphene tolerance was indicated in the Yakima Valley and Kern County. *Lygus elisus* and other congeners may be involved in the resistance to DDT and toxaphene.

Triatoma infestans. The development in this cone-nosed bug of DDT resistance within 3–4 months was reported from Chile in 1948 (358); however, this species is so normally refractory to DDT that BHC is used to control it. By 1953 no BHC resistance had developed in South America (403).

Cimex lectularius (bedbug). DDT resistance was first noted at Pearl Harbor, Hawaii, in 1947 (234), three years after the chemical was introduced. It appeared in an infestation at Columbus, Ohio, in 1952, which showed 0% mortality on deposits that normally killed 100% (6). Other 1952 records of resistance include Chicago, southern Indiana, Salt Lake City, Belgian Congo (447), Israel (267), Korea, and Laurion, Greece (184). In 1953 bedbugs as DDT-resistant as the Columbus strain were found at Kuskoa, Japan, and on the *U.S.S. Salerno Bay* (293a), and a population resistant to both dieldrin and DDT at Gaeta, Italy (351). In 1955 resistance was reported from Denver, Philadelphia, and Dallas (458) in the United States and from Tehran (340). There are no reports of DDT resistance in South America (463), with the exception of Cayenne, French Guiana, into which DDT-resistant bedbugs from the island of St. Lucia have been recently introduced (148). Resistant strains reared in the laboratory without DDT lost their resistance quite rapidly (293a).

BHC resistance was observed in Israel in 1956, being 160 times at Beth Shaan, and 40 times at Tiberias, that of a normal laboratory strain (111).

Cimex hemipterus. DDT resistance in this tropical bedbug appeared in 1952 at Feng-Shan, southern Taiwan, in barracks sprayed for two years (95). They showed 0% mortality to DDT exposures twice as intense as killed 100% of a normal population, but were susceptible to BHC. In 1952 DDT resistance was also observed in Kowloon, Hong Kong, although DDT had been seldom used; again the insects were susceptible to BHC (428). A similar resistance pattern has also appeared in Singapore (80). In 1955 DDT resistance was reported to be quite widespread in coastal Bombay State, with 0% mortality to exposures that killed 70% in 1952 or in untreated areas (413). By 1955 BHC was becoming ineffective. and by 1956 dieldrin was failing.

Dieldrin resistance independent of DDT resistance developed in *C. hemipterus* at South Pare, Tanganyika, in 1957 (447b). It also developed in *C. rotundatus* at Pala, Upper Volta, French West Africa (190b).

Erythroneura variabilis (grape leafhopper). DDT resistance was noted in 1951 near Phoenix, Arizona, as a failure of the control obtained in the six previous years; these insects proved immune to deposits that killed normal strains in 1 hour (27). Simultaneously it appeared in the Coachella Valley of southern California, where the leafhoppers were found to be 6 times as DDT-resistant as normal; DMC and other synergists failed to restore control (27).

Erythroneura elegantula. DDT resistance appeared in 1951 in certain vineyards of Tulare County, California; 10% dusts gave poor control where 5% dusts had formerly been very effective. A sample of insects taken from Woodlake showed only 20% mortality to deposits that killed 95–99% of normal leafhoppers. The resistance extended to Perthane, but not to malathion (460).

Erythroneura lawsoniana. DDT resistance appeared in 1953 in an orchard near Paducah, Kentucky, where this chemical had formerly given good results (188). DDT resistance in the apple leafhopper (presumably *Empoasca maligna*) has been reported in 1954 for some areas of the United States (258).

Chromaphis juglandicola (walnut aphid). Parathion resistance was first noted in 1953 near San Jose, California, as a threefold increase in the field dosage required to control. Laboratory comparison showed the LD_{50} to be 7 times that of a normal population (331).

Aphis gossypii (cotton aphid). HCN tolerance of a slight order was induced by 7 generations of heavy laboratory pressure (52). BHC resistance was suspected in many areas of the United States as a contributing factor to control failures in 1953 (258). In 1957 resistance was suspected in Alabama, Arkansas, Louisiana, and Mississippi (153a). But a South Carolina strain reported to be BHC-resistant was no less susceptible than a normal Texas strain (229). DDT-resistant strains of *Macrosiphum solanifolii* (potato aphid) were reported present in some areas of the United States in 1954 (258).

Myzus persicae (green peach aphid). Resistance to parathion, malathion, and TEPP appeared in orchards at Rock Island and Trinidad, Washington, in the spring of 1953. Field tests confirmed this resistance, but tests on a greenhouse-reared colony did not. The same phenomenon appeared at Yakima in 1952 (9). Parathion resistance was also reported for *M. cerasi*, *Sapaphis pyri*, and *S. plantaginis* in Switzerland in 1954 (499).

Aspidiotus perniciosus (San Jose scale). Resistance to lime-sulfur sprays was first noticed in the Clarkson Valley, Washington, in 1908. A sample tested in 1913 showed only 4–17% survival to sprays that killed 100% in surrounding districts (319). Similar resistance was noted in southern Illinois in 1920 (146). Both strains were susceptible to oil-emulsion sprays.

Saissetia oleae (black scale). HCN resistance was first noted at Charter Oak, California, in 1912 (513), and by 1938 had spread throughout Los Angeles County. The resistant strain showed 9–25% survival to vapor dosages of HCN that killed all but 1% of normal populations (407).

Coccus pseudomagnoliarum (citricola scale). HCN resistance was first observed near Riverside, California, in 1925, and occurred over the entire species range by 1938. Resistant samples showed only 3–58% mortality to doses that killed 49–94% of the normal stock (407).

Aonidiella aurantii (California red scale). HCN resistance was first noted at Corona and Orange, California, between 1912 and 1914. It allowed approximately 20% survival to doses that killed all but 1% of the normal strain, according to laboratory tests in 1922 (407) and field tests in 1925 (513). It continued to spread, even when HCN fumigation became rare, so that by 1938 it occurred all around Los Angeles (407) and by 1946 characterized a large part of the red scale population in southern California (125). The level of resistance also increased further from 1935 to 1945 at Corona with one fumigation per annum, and laboratory pressure for 43 generations raised it a further 5 times to the superresistant level (521). Material collected in 1935–38, when reared free of HCN, retained its resistance for 6–10 years (521,523) or 70 generations (279), and crossing these inbred strains did not give any greater resistance (523).

The resistant strain shows no abnormalities in morphology, developmental rate (524), mortality at unfavorable temperatures, or reproductive rate (357). Although just as susceptible as normal to ethylene dibromide (280), parathion (328), and mineral oil (278), the adults were more resistant to methyl bromide (407,520) and to ethylene oxide (407) and slightly more tolerant than normal to rotenone

(106). Superresistant, resistant, and susceptible strains show the same order of response to H_2S as to HCN, but the reverse order to nitrogen anoxia (524).

Resistant strains can acquire the condition known as protective stupefaction, which allows them to develop tolerance of lethal concentrations within 2 minutes of exposure to sublethal doses; the normal stock requires a period of 20 minutes to develop this tolerant condition (277,408,522). It was at first considered that resistant scales responded to HCN by keeping their spiracles closed for a half hour, whereas susceptibles closed them for only 1 minute (195). But although the resistants generally closed them earlier, they reopened them during the second half hour, by which time the susceptibles usually had closed their spiracles again (519). Moreover, the differential between strains shows up for short-period fumigation even before the spiracles close (408). No more HCN is absorbed into susceptibles than into resistants (281).

It has recently been found that the more HCN-resistant a strain is, the more resistant it is to the depression in respiration which HCN induces (524). That resistants are less dependent on the HCN-sensitive cytochrome system was also suggested by their containing less Cu and more reducing substances, but there was no difference in the Fe content (181). The genetic origin of HCN resistance was found to be a sex-linked gene without dominance, so that females are resistant (RR), intermediate (Rr), or susceptible (rr), and the heterogametic males are either resistant (R) or susceptible (r) (124,523).

Pieris rapae (imported cabbage worm). DDT resistance was encountered in an area of 6–8 miles radius in Kenosha and Racine counties, Wisconsin, late in the summer of 1951, after five years (15 generations) of successful control (302). Dust treatments that killed almost 100% in other areas failed to kill more than 50% in this area, the figures indicating a 60-fold resistance as compared with a 1944 New York population. Control failures had appeared by 1952 on Long Island (207), by 1953 in Florida (205) and Connecticut (489), and by 1954 in western New York (207). In Connecticut it was found that resistance had increased 10 times even where control failures had not yet developed. In western New York adequate control in 1954 required 20 times as much material as in 1944. Control failures in three districts of New South Wales in 1950 were found to be due to inadequate dust coverage (384). The DDT resistance in Wisconsin extended to methoxychlor, DDD, and Perthane, but not to Dilan, dieldrin, and endrin (302,489).

DDT resistance has also developed in *P. rapae crucivora* in the Kanto and Tohoku areas of Japan. The resistant larvae can convert more than 80% of the absorbed DDT into DDE within 48 hours (258a).

Protoparce quinquemaculata (tomato hornworm). This species is characteristically highly DDT-susceptible, in contrast to *P. sexta* (461). But repeated control failures with DDT and other insecticides in Florida in 1951–52 forced many growers to substitute parathion (16).

Trichoplusia ni (cabbage looper). DDT resistance in western New York State was very much greater in this species than in *Pieris.* By 1954 even 8% DDT dust allowed more than 25% survival (207). The steady increase in resistance by 1955 dictated that DDT be discontinued, endrin being the most effective substitute (303). Resistance in this species has also been reported as present in California and Louisiana (153a).

Alabama argillacea (cotton leafworm). Toxaphene resistance began to appear in Texas in 1951. Larvae collected at College Station and Port Lavaca were 4 times as resistant in 1952 and 10 times as resistant in 1953 as in 1946 (229). Evidently the resistance originated in Central or South America, which annually supplies the populations of North America; indeed, control failures with chlorinated hydrocarbons appeared in Venezuela in 1951, and resistance became general there by 1953 (365).

Heliothis armigera (cotton bollworm). A decline in susceptibility of the cotton bollworm to chlorinated hydrocarbons was reported from North Carolina in 1953 (16). However, the susceptibility at College Station, Texas, remained the same in 1954 as when DDT and toxaphene were introduced 8–10 years previously (229). Resistance was reported in 1957 for the beet armyworm, *Laphygma exigua,* in California, and for the cotton leaf perforator, *Bucculatrix thurberiella,* in the Imperial Valley (153a).

Anarsia lineatella (peach twig borer). Arsenic resistance was discovered in 1944 in a small area near Empire, California. The usual two cover sprays of basic lead arsenate resulted here in fruit losses of 20–50%. Tests in 1948 showed that 20% of the larvae could penetrate spray coatings, where normally only 3% could do so (469).

Carpocapsa pomonella (codling moth). Arsenic resistance reached the point in 1928 in the Grand Valley of Colorado where 10–12 cover sprays of acid lead arsenate were required to give the control given by 2 sprays annually in 1900 (220). It was noted in one orchard in the Hudson Valley, New York, in 1930, and by 1945 had spread over the western part of the state (382). Increase of this type of resistance was noted in the Yakima Valley, Washington, from 1930 to 1933 (486), and in the Shenandoah Valley, Virginia, between 1928 and 1943 (223). Arsenic resistance developed in certain orchards of the Elgin district of western Cape Province, South Africa (467c). Resistance to basic lead arsenate deposits appeared in California walnut orchards in 1943 (409).

The resistance of the Colorado strain extended to deposits of cryolite, barium fluosilicate, or rotenone. It was found that the larvae were larger and more resistant to starvation and desiccation than a normal Virginia strain (222). Hybrid larvae were intermediate in resistance, and successive hybrid generations continued intermediate without noticeable segregation; back-crosses gave progeny intermediate again between the hybrid and parental strain (221). This resistance evidently derives from the ability to live longer to find a poison-free spot on the apple, for once they ingested the poison the Colorado larvae were just as susceptible as the normal strain (203).

DDT resistance was first noted in 1951 at Hamilton, Ohio, where in 1953 the DDT spray schedule that allowed only 0.5% infestation in 1945 now permitted 95% infestation (110). Another control failure was noted in 1952 near Lockport, New York; field and laboratory tests indicated that the resistance here was more moderate (170). DDT failed to provide control in 1953 at Gleed, Washington, and laboratory tests showed that larvae from Gleed and Yakima were as resistant as the Hamilton strain. The control obtained with DDT noticeably deteriorated in 1955 in southern Indiana, Illinois, and Kentucky, and failed in 1953 in one orchard near Henderson, Kentucky; but laboratory test of the larvae found them to be more susceptible than the Hamilton strain (187). DDT resistance is also present in the Elgin district, Cape Province, South Africa (50,357a), and in South Australia (294a).

Argyrotaenia velutinana (red-banded leaf roller). DDT resistance first appeared at Sodus, N. Y., in late 1954, and by 1956 the control failures had extended to 5–10% of the apple orchards in western New

York. Tests made in 1956 with larvae on leaves dipped in various concentrations of DDD in aqueous suspensions showed the LC_{95} for two strains at Sodus to be 10 times that obtained for a strain from a Chalupa orchard where DDD had not yet been used (169a).

Earias insulana (spiny bollworm). Endrin resistance appeared in 1956 on cotton in the Beth Shaan area of Israel, after three years of fortnightly treatments. Samples from four different villages showed only 17–25% mortality to endrin deposits that killed 72–90% of larvae from untreated areas (13).

Plutella maculipennis (diamondback moth). DDT resistance was discovered in 1951 in cabbage fields near Bandung in Lembang, Java, where heavy applications had been made since 1948; the caterpillars remained highly susceptible elsewhere on the island. Laboratory tests proved this strain to be 7 times as resistant to DDT dust as a normal population and to show mortalities of about 5% to dust deposits that normally killed 94% (5,233). The Lembang strain was slightly less susceptible to BHC and toxaphene, which still controlled it.

Pyrausta nubilalis (European corn borer). Although DDT resistance has not been reported for this species, fifth-instar larvae studies at Ames, Iowa, were appreciably tolerant of DDT and converted it to significant amounts of DDE (286).

Conoderus falli. Chlordane resistance of this wireworm was first noted in 1955 in Charleston County, South Carolina, and intensified in 1956. Upon laboratory tests this strain showed less than 30% mortality to soil concentrations of chlordane which killed 100% of a seldom-treated population. The resistance extended to aldrin, dieldrin, and heptachlor, but not to DDT. The Gulf wireworm, *C. amplicollis,* also present in the area, showed no tendency to resistance (417).

Tribolium confusum (confused flour beetle). Offspring of survivors of a given dose were found to be 1.6 times as HCN-tolerant as those destined to be victims. The differential of the two groups was inherited, and the more tolerant group showed a 20% higher oxygen uptake (172). DDT tolerance has been induced by 10 generations of laboratory pressure, which has increased the LT_{50} by 5 times (228). Of six strains of *Tribolium castaneum* (red flour beetle) tested in England, the extremes differed by 4 times in susceptibility to γ-BHC and pyrethrins (217).

Leptinotarsa decemlineata (Colorado potato beetle). DDT resistance has been reported as developing in New York State (382) and elsewhere in the United States (258).

Epilachna varivestris (Mexican bean beetle). Rotenone tolerance first appeared in New York State in 1949, where 1.0% dusts were needed for the control afforded by 0.75% dusts during the previous 20 years (382). In 1952 it was detected in Connecticut, where the concentration of dusts necessary to obtain 90% control was 5 times as great as in 1942, but did not require changing the insecticide (482). Control failures began to be observed in 1951 around Mills River, North Carolina. Field tests showed only 70% control here with doses that controlled 95% elsewhere; laboratory tests in 1954 showed the Mills River strain to be 5 times as resistant as normal, but in this case it was enough to dictate a change in insecticide (57). This species is normally DDT-tolerant and can detoxify DDT *in vivo* to DDE, which is converted to other compounds before excretion (236,463).

Epitrix cucumeris (potato flea beetle). DDT resistance first appeared in northern Indiana and in Connecticut in 1951, five years and 5 generations after DDT was introduced. In 1952 it was general in northern Indiana, and the situation had become serious in Connecticut (173,259). Tests at Windsor and Windsorville, Connecticut, in 1953 and 1954 showed that the DDT schedule now reduced damage by only 17% as against 73% in 1946 (258). Dieldrin and endrin were the most effective substitutes (259).

Anthonomus grandis (boll weevil). Toxaphene resistance did not develop in Texas between 1945 and 1953, the susceptibility to BHC also remaining normal (229). But in 1955 a high degree of resistance was reported from northeastern Louisiana and the Ouachita and Red River valleys and the Macon ridge in that state, involving toxaphene, dieldrin, heptachlor, endrin, and BHC; topical application tests indicated the population at St. Joseph to be about 100 times as endrin-resistant as a reference colony from Baton Rouge (432,432a). Some reports implied the apparent resistance was due to late-season tolerance and a very heavy infestation (7). But in 1957 the boll weevil was found to be resistant in Arkansas, Louisiana, Mississippi, South Carolina, and Texas and suspected in parts of Alabama. It was most serious in the Mississippi Delta, where control decayed in 1953 and failed in 1954 (153a). Although the seasonal increase in the normal insecticide tolerance of this species is associated with an increase in fat content, the St. Joseph resistant strain developed no more fat than the normal (56a).

Sitophilus granarius (granary weevil). Tolerance of methyl bromide sufficient to require twice the usual dosage was induced by 14 generations of laboratory selection of two Canadian strains (343). However, an English field strain was found to be normally about 5 times as resistant to pyrethrins as a laboratory strain (217).

Scolytus multistriatus (smaller European elm bark beetle). DDT resistance, extending also to methoxychlor, was reported in 1951 from an area treated with DDT for several years (58).

Macrocentrus ancylivorus. DDT tolerance has been induced in this ichneumonoid parasite of the oriental peach moth by 18 months of selection, which increased the LD_{50} by 5–6 times (397,505). With continuing DDT pressure, the tolerance increased to 12 times by the 19th generation, only to decline to 7 times by the 71st generation of pressure and to disappear completely in 14 subsequent generations of relief from DDT (426a).

Phlebotomus pappatasii. Contrary to a report detailed from Issaris by Brown to Hess (208) this species of sand fly has not developed DDT resistance in Greece (184). In Italy also DDT has remained particularly effective (346).

Psychoda alternata. DDT resistance in larvae of this filter fly developed in Illinois in 1949, after two years of susceptibility; chlordane resistance developed in the subsequent year (64,65). BHC resistance of *Psychoda* larvae in England developed in 1953, after two years of susceptibility, so that heavy doses became necessary for control (104).

Glyptotendipes paripes. BHC resistance of larvae developed in 1953 in the lakes of Polk County, Florida, within six months of the first application. EPN resistance appeared in two lakes in 1955, one year after the first application; this was confirmed by laboratory test (275).

Culex fatigans (*quinquefasciatus*) (southern house mosquito). BHC resistance of larvae appeared in 1953 at Georgetown, Malaya, where it was 10 times the normal after three years of treatment (416); and at Kumbakonam, Madras, where it was 6 times the normal after 20 months of treatment (410). It extended to dieldrin but not to DDT (415). It has also developed in California (169), French Guiana (147,148a), and Hong Kong (428). Dieldrin resistance developed within three months of use at Singapore (102). Larvae in Ceylon developed resistance to BHC and dieldrin (96), on Okinawa to dieldrin and chlordane (158a), and in Ecuador to BHC and chlordane (266). The BHC resistance present at Georgetown was absent from the untreated Balik Palau only 10 miles away, and it was largely lost in 10 generations of normal laboratory culture (415).

Dieldren resistance of adults, extending to the larvae, developed in 1957 at Ubai and Kuantan, Malay (488a), and at Pala, Upper Volta, French West Africa (190b).

Considerable DDT tolerance already pre-exists in larvae of *Culex fatigans*. The LC_{50} levels in Malaya are naturally 0.15–0.25 p.p.m., five to ten times that for *Anopheles* or *Aedes* (415,488). Yet this natural tolerance evidently can be increased to resistance; at Puerto Cabello, Venezuela, in 1956 it had reached an LC_{50} of 5.0 p.p.m. (42) and on La Réunion after four years' treatment the mortality was only 15% to doses that killed 98% in untreated areas (189). DDT resistance in larvae has also been reported from Taiwan (287b), Manila (130), India (260), Ceylon, and Ecuador; but in California the LC_{50} levels in treated areas were not significantly above the natural tolerance (169).

Considerable natural DDT tolerance also exists in adults. The LC_{50} by Busvine-Nash test (83) for *C. fatigans* in Malaya was over 5% DDT, more than 3 times that of any other species tested (488). Adult mortalities in huts sprayed at 200 mg. per square foot in Kenya were only about 10% as compared to approximately 60% for the local *Anopheles* (115). Nevertheless, DDT pressure can increase this tolerance; adults from a village treated for six years in Delhi State showed only 9% mortality to deposits that killed 90% of those from an untreated village (381). When strains from either village were submitted to laboratory pressure for 40 generations, that from the untreated village increased its tolerance by 6 times, that from the treated one increased it by 1.6 times (442). Considerable increase of tolerance developed in one Indian strain simply from 10 months of insecticide-free rearing (364). Adult pressure for 10 generations on a Venezuelan strain increased the tolerance by only 25%, and an unusual number of gynandromorphs appeared (43). In the many citations of adult DDT resistance of *C. quinquefasciatus* in South America (208,403) there is no proof that the natural tolerance increased, whereas there are three statements that it did not (147,161,439).

Culex pipiens (northern house mosquito). DDT resistance of larvae in excess of normal levels was observed in Toledo, Ohio, in 1952

(209), Boston and Cambridge, Massachusetts, in 1953 (11), and Hoboken, New Jersey, in 1956 (67); but it was not present in southern Ontario after as much as eight years of DDT treatment (61).

Culex molestus. DDT resistance of adults developed in Latina, Italy, in 1947 after one year of house spraying (349). Their capacity to survive deposits of 200 mg. per square foot persisted for 8 generations of laboratory culture. Similar resistance evidently appeared in 1948 in Greece (184) and Morocco (157). DDT pressure applied to the larvae for 15 generations did not change the LC_{50} of a laboratory colony(445).

Chlordane resistance developed in the Latina population by 1950, following susceptibility for two years; a similar picture developed with BHC (346). Dieldrin resistance followed DDT resistance in Jerusalem, Israel (174a).

Culex tarsalis. DDT resistance in larvae was first noticed in Kern County, California, in late 1947 (451). Tests performed in 1951 showed the resistance in the San Joaquin Valley to range from 6 to 10 times the normal (169). The level doubled again by 1955 and was no less in regions outside the mosquito control districts (166). DDT resistance appeared in 1956 at Oakridge, Oregon (132a,167).

Toxaphene resistance appeared at two points in Kern County in 1951, two years after the chemical was introduced. This resistance was 10–30 times the normal and extended to aldrin, dieldrin, heptachlor, and γ-BHC (169).

Malathion resistance appeared in 1956 in Fresno County, California, five years after the introduction of organophosphorus insecticides. Larvae in treated areas were 20–30 times as resistant as those in untreated. The resistance did not extend to parathion, but the adults were also resistant to malathion (168).

Aedes aegypti (yellow fever mosquito). DDT resistance of larvae appeared in the Plum Mitan area of Trinidad in 1950 (165). When tested in 1955, these larvae showed an LC_{50} of 4.0 p.p.m. (135); with laboratory strains showing LC_{50} values of 0.004–0.05 p.p.m. (78,379), this may be deemed resistance about 500 times the normal. In 1956 DDT resistance was reported in eastern Venezuela, with an LC_{50} of 0.4 p.p.m. at Carupano and 2.0 p.p.m. at Caracas (42). It also developed at Cucuta, Colombia (383a), and Port-au-Prince, Haiti (80a). Control failures with DDT also developed at Paramaribo, Dutch Guiana, in 1953 (403), and Ciudad Trujillo, Dominican Republic, in 1954 (383). The Trinidad resistant strain was also resistant to DDD and to DMC-DDT mixtures. The adults showed only 4% mortality to DDT exposures which normally killed 76%, but addition of DMC increased this mortality (135).

DDT pressure for 15 generations on larvae of a laboratory strain did not increase the LC_{50}; but pressure on a strain from Klang, Malaya, already 3 times more tolerant and abnormally small, increased this tolerance by 7 times again in 8 generations (445). Larvae of the Trinidad strain convert large amounts of DDT to DDE *in vivo*, while a normal strain does not (62). But *in vitro* with glutathione as activator no DDE was formed by tissue extracts (60).

Larvae of the Trinidad strain were of normal susceptibility to BHC, dieldrin, and Strobane. House spraying for two and a half years with BHC did not decrease the susceptibility of adults in Nigeria (81). But γ-BHC pressure on larvae of a laboratory strain for 15 generations doubled the LC_{50} to γ-BHC, dieldrin, and DDT (445).

Aedes sollicitans and A. taeniorhynchus (salt marsh mosquitoes). DDT resistance was first noted in *A. sollicitans* at Cocoa Beach and East Hollywood, Florida, in 1948 after three years of spraying (37,87), and was reported from other counties in Florida in 1949 (253). Tests of *A. taeniorhynchus* from Cocoa Beach indicated the larvae to be at least 10 times, and the adults 6–8 times, as DDT-resistant as those from untreated areas. The resistance extended to DDD but not to BHC or dieldrin (122,123). The DDT resistance in larvae of *A. sollicitans* at Bombay Hook, New Jersey, was 6 times that in untreated salt marshes (114a).

BHC resistance was first noted at Cocoa Beach in 1951 (244) and proved in 1952 to be at least 8 times the normal (240). Resistance of 3–10 times the normal was also shown to dieldrin, toxaphene, heptachlor, aldrin, EPN, and malathion (240).

Aedes nigromaculis and A. dorsalis (irrigation water mosquitoes). DDT resistance was first noted as a lack of control of larvae in Kern County, California, in late 1947. Tests of *Aedes nigromaculis* larvae in Tulare County in 1949 showed them to be about 10 times as resistant as normal (49). Tests in 1951 in six counties showed *A. nigromaculis* and *A. dorsalis* to be 3–8 times as DDT-resistant in treated areas as in untreated ones (169). By 1955 the DDT resistance of *A. nigromaculis* in untreated areas equalled that in treated areas, presumably as a result of the use of agricultural insecticides.

BHC resistance was absent in 1951, but a few samples of each species showed resistance to toxaphene (169). As of 1956 *A. nigromaculis* had not developed resistance to the organophosphorous compounds employed in the previous five years (168).

Aedes vexans, canadensis, etc. Citations of resistance in *Aedes vexans* (209), *A. punctor* (433), and *A. communis* (433) have not been confirmed in fact. Larvae of *A. canadensis* and *A. fitchii* in areas of southern Ontario treated for periods up to eight years were no different in susceptibility from those in untreated areas (61).

Psorophora confinnis and P. discolor (rice field mosquitoes). Dieldrin resistance of *P. confinnis* larvae appeared in certain rice fields of Bolivar County, Mississippi, in mid-June, 1954, only two months after dieldrin had been successfully introduced there. Adults from these larvae showed 17% mortality to doses that killed 87% of those from fields not larvicided. It is concluded that this resistance had been predisposed by the use of dieldrin, toxaphene, and BHC as agricultural insecticides in the area. Dieldrin resistance of *P. discolor* followed in April, 1954 (317).

Anopheles quadrimaculatus (common malaria mosquito). Dieldrin-resistance of larvae in Bolivar County, Mississippi, was first noted as a lack of control in 1953 (317). Adults of the population tested in 1955 showed 0% mortality to dieldrin deposits at 200 mg. per square foot, which killed 100% of a natural laboratory strain. Similar reduced mortality was shown to BHC (3% vs. 67%) and to chlordane (5% vs. 44%), but not to DDT (318). It was concluded that this resistance had been induced by agricultural insecticides.

An increase in DDT tolerance of larvae was detected in the Tennessee Valley in 1950, after six years of spraying; larvae from treated areas showed a slightly but significantly lower mortality than those from untreated (296). In 1951 larvae collected from treated areas (261) showed an LC_{50} approximately 20 times that found in 1944 (329) and a dosage of 0.1 lb. per acre was needed where 0.05 lb. per acre had sufficed seven years before. Contamination of the area with chlorinated hydrocarbons from crop dusting had induced a notable increase in tolerance of larvae that were field-collected and then bred for bioassay purposes (261). However, the dosage of 0.1 lb. per acre remained effective until at least 1953 (156). The field evidence therefore indicates that nine years of larviciding and crop dusting has slightly increased the DDT tolerance of *A. quadrimaculatus.*

Heavy DDT pressure on larvae of a strain from Kentucky Reservoir increased the LC_{50} by 3 times in 5 generations, decreasing the frequency of the most susceptible phenotypes (204). Intense DDT pressure on this strain for 12 months increased the larval tolerance only 2.5 times (441).

Adults tested in 1950 were very slightly less susceptible to DDT deposits than those from untreated areas (296), and in sprayed rooms the mortality was less than in 1944 (329). But exactly duplicated tests in 1955 showed that the window-trap mortality and the speed of kill were essentially the same as in 1944 (452). When adults were selected by DDT pressure in the laboratory, their offspring were less susceptible than normal, but thereafter there was no further decrease for 4 generations. When selection was relaxed, the next generation of adults showed the original susceptibility. Evidently the offspring had received maternal effects for tolerance, but they cannot increase the range of maternal effects. Similar results were obtained with methoxychlor, but no changes at all appeared with DDD, BHC, chlordane, or aldrin (136). Selection of adults at the Wilson Dam laboratory for 18 generations produced little change in their susceptibility. Adults and larvae can produce DDE from DDT, but those collected from treated areas produce consistently more than those from untreated (156).

Anopheles gambiae. Dieldrin resistance was discovered in 1955 in western Sokoto Province, northern Nigeria, 18 months after the inception of house spraying with dieldrin. The LC_{50} of the adults was 8 times that of normal *A. gambiae* from Lagos and elsewhere (133). Eggs collected at Ambursa were sent to London, and 5% hatched to found a colony characterized by longer adult life and greater ease of culture than normal. On testing, adults of this colony showed an LC_{50} to dieldrin of 800 times the normal, with similar high resistance to chlordane and related compounds, and 26 times the normal resistance to γ-BHC. The susceptibility to DDT was unchanged, and the tolerance of malathion and pyrethrins was only twice the normal (119). Crosses of the resistant Ambursa strain with the susceptible Lagos strain gave F_1 hybrids that were intermediate in resistance, all being killed by 4% dieldrin but all surviving 0.33% dieldrin. When back-crosses were made with each of the parental strains, the offspring in either case were composed of hybrid and parental types in 50:50 ratio. This is proof of dieldrin resistance being produced by an allele of a single gene, all individuals of the Ambursa strain being homozygous for it (120). The resistant Ambursa strain was found to absorb just as much γ-BHC as the normal strain and to convert no more of it to water-soluble metabolites (56).

Dieldrin-resistant populations have subsequently been found to have developed at Kano, northern Nigeria (132b), Abidjan and Aboisso, Ivory Coast (1), Bobo Dioulasso, Upper Volta (189a), up-country Liberia (60a), and Freetown, Sierra Leone (132b).

Behavioristic resistance to DDT was reported by Muirhead-Thompson (352–354), but this was not a case of developed resistance over generations of selection. He observed that *A. gambiae* and *A. melas* in mud huts treated with DDT in kerosene at Lagos (352) and *A. gambiae* and *A. funestus* in mud huts treated with DDT wettable powder at Dar-es-Salaam (354) left the walls without acquiring a lethal dose. However, this effect was evidently due to the DDT deposit being insufficient, owing to absorption from oil solution at Lagos, and to the coarseness of the wettable powder at Dar-es-Salaam (116,506). Subsequent workers have found that *A. gambiae* shows high mortalities inside treated huts and in window traps (182,214). It has been found in vector control programs on Mauritius (127) and La Réunion (190) that the surviving population is largely exophilic, but there are no grounds to consider that this exophily was developed as a change in habits.

Anopheles sacharovi. DDT resistance of this species in Greece, where control operations had commenced in 1946 with great success, gave its first premonitions in 1949 with the mosquitoes escaping from the houses to recover outdoors (184). From 1950 to 1953 increasing numbers were found in sprayed houses, and the 24-hour mortality of specimens collected therefrom became progressively less (159,160,-290,291). From 1953 to 1956 the LC_{50} values by Busvine-Nash test of adult females in the Pelopponesus were successively 1.2, 2.6, 3.0, and 4.0% (76,185,193) as compared to 0.2% for a population of *A. sacharovi* in an untreated region (8). Already by 1953 DDT resistance had become general in Greece (35), more pronounced in some areas than others (290) and particularly in the rice-growing region of Pelopponesus where DDT had also been used as a larvicide. However, DDT resistance was not evident in larvae at that time (35). In 1953 two isolated villages of northern Lebanon showed DDT-resistant populations, with LC_{50}'s of 1.7%, and mortalities of only 44% at levels that killed 98% of those from a neighboring village

(155). Increased DDT tolerance has been detected in the Kazerun region of Iran and strong DDT resistance near Tarsus, Turkey (526).

Chlordane resistance of adults appeared at Skala, Lakonia, in 1952 (160), three years after chlordane was first used; when dieldrin was employed in 1953, it encountered resistance in that year (34,290). Chlordane resistance of larvae suddenly appeared in 1956 in four districts of Greece, where DDT had been used since 1946 and chlordane since 1955; dieldrin resistance was even more marked, although this insecticide was not used until 1956. The LC_{90} values obtained at Astros, Arkadia, were 7 p.p.m. for chlordane and 22 p.p.m. for dieldrin, while those for DDT remained normal (36).

Anopheles sundaicus. DDT resistance was discovered at four points on the north coast of Java: Tandjung Priok and Tjirebon in 1954 (105), Semarang in 1955 (97), and Soerabaya in 1956 (96). Later it was found in the region of Akyab, Burma (60a). Adults at Semarang were 18 times as resistant to DDT as the normal (117), while remaining susceptible to dieldrin and γ-BHC. DDT had been used as a larvicide in these rice-growing areas before the inception of house spraying. In the Tjilatjap area on the south coast *A. sundaicus* reacted by avoiding DDT-sprayed walls (227); since this response occurred when a village was sprayed for the first time (485), there is no reason to consider it as development of behavioristic resistance. The DDT resistance of the Soerabaya strait is due to a single recessive gene (120a) which promotes the detoxication of DDT to DDE (60a).

Anopheles stephensi. DDT resistance was indicated as a lack of control in 1953, and confirmed by test in 1955, at two oases in the region around Dhahran, Saudi Arabia, where house spraying had commenced in 1947. The adults were at least 10 times as resistant to DDT as those from untreated areas, while remaining as susceptible as a laboratory colony to dieldrin and γ-BHC (111a,118). Subsequently DDT resistance was found to be present throughout the range of the species around the Persian Gulf, from southern Iraq and Iran east to Bandar Abbas (60a). DDT resistance of larvae was discovered in 1955 at Erode, south India, where wells had been treated with DDT since 1947; only 44% mortality was shown at dosages which killed 100% of those from an untreated area (411). Larval pressure for 12 generations in London on a laboratory colony originating in Delhi raised the larval and adult tolerance only slightly by removing the most susceptible phenotypes, and so did adult pressure; the increase disappeared on relaxation of pressure.

Anopheles maculipennis. DDT resistance of adults in Greece has been observed in sprayed premises since 1953 (35,290), but has not been confirmed by test. In villages north of Isfahan, Iran, sprayed for five years, adults showed LC_{50} values of over 4.0%, while the LC_{50} was well below 2.0% for adults in the unsprayed city of Isfahan itself (525).

Anopheles superpictus. DDT resistance of the species in Greece was reported in 1953, on the basis of the return of adults to sprayed houses (34,35). It is also claimed that this resistance extends to chlordane and dieldrin (35,290). However, the only test figures available, namely, from the unsprayed village of Triada in the heavily sprayed region of northern Greece, show the LC_{50} to be less than 0.5% and indicate that no resistance has developed there (185,186).

Anopheles subpictus. DDT resistance was observed in 1955, after seven years of spraying, in the village of Jwalaheri, Delhi State. Tests of adults performed in 1956 by the Busvine-Nash method showed an LC_{50} of 3.1% with 24 hours' exposure, as compared to the normal LC_{50} of 0.4% for 1 hour's exposure (443). DDT-resistant *supbictus* in the neighboring village of Nanglisahidan were very susceptible to diazinon residual sprays (443a). Dieldrin resistance was detected in dieldrin-sprayed areas near Tjirebon and Semarang, Java, and in DDT-sprayed areas in Bombay State, India (60a).

Anopheles atroparvus. DDT resistance has apparently not been developed by the species in Italy, Spain, and Holland. On a laboratory colony at Rome, larval pressure has raised the larval resistance 9 times in 8 generations, and 16 generations of pressure on larvae and adults has produced a strain that is 50% immune to exposure to 3% DDT for 24 hours. This colony shows a dimorphism of chromosome III, 47% of the individuals being homozygous for an inversion between sections 44 and 48. The inversion homozygotes all survive a dosage of 0.5 p.p.m. DDT, which kills all the normal homozygotes. DDT pressure for 3 generations increased the proportion of inversion homozygotes in the population to 63% (113).

Anopheles culicifacies. The DDT susceptibility of this species has remained normal, despite up to eight or ten years of spraying operations in Ceylon, in Delhi State (378,381) and Bombay State (412). *A. fluviatilis* has also responded normally in these regions; 47 generations of laboratory pressure with DDT gave a slight increase in DDT tolerance, not detectable until the 29th generation (341). *A. pseudopunctipennis* in Mexico showed no change in susceptibility during five years of DDT spraying (128,152). *A. minimus flavirostris* in the Philippines has maintained precisely the same levels of DDT susceptibility during the operations from 1954 to 1956, as indicated by the speed of knockdown (322).

Anopheles albimanus. Behavioristic resistance to DDT evidently developed in the villages of Gatuncillo and Santa Rosa, Panama, where control failure became evident in 1952. Engorged females showed only 65% mortality in sprayed houses in 1952, as against 96% in 1945. Yet when confined to DDT-treated papers in the Busvine-Nash test, they were just as susceptible as a laboratory colony (474,475). The adults in these villages characteristically were more phototropic and spent less time resting on walls than the normal. Subsequent laboratory tests showed that these adults were more quickly irritated by DDT than the normal (60b). DDT pressure applied to a laboratory strain for five years did not increase the physiological resistance of this species (475), but did decrease its irritability to DDT (60b).

Anopheles darlingi. Behavioristic resistance to DDT was reported to have developed in 1949 at Engenheira Dolabela, Minas Geraes, Brazil, three years after house spraying was commenced. Adults had assumed the habit of resting on outside walls rather than inside (the ratio being 48.3, as against 2.0 for unsprayed villages,) their numbers were no longer reduced, and malaria transmission was resumed (68, 70). There was evidence that the population was now more readily driven out of the sprayed houses than previously (69).

Dacus dorsalis (oriental fruit fly). Some DDT tolerance was induced by the 4th generation of laboratory pressure, but did not increase thereafter (16,89).

Rhagoletis completa (walnut husk fly). A cryolite resistance was noted in southern California in 1943 which did not exist nine years before (409).

Leptocera hirtula. DDT resistance in adults of this Borborid fly developed after two to three months' control by residual deposits. BHC resistance then developed after a few months of the use of BHC (416).

Drosophila melanogaster (pomace fly). HCN tolerance was induced by laboratory selection with HCN only very slowly (29) and to a limited extent (52). The most HCN-tolerant strain obtained was more tolerant than normal to cold and ether narcosis, but was susceptible to DDT and tartar emetic (29). The HCN-tolerant Bayfordbury strain developed by 46 generations of selection showed 25–30% mortality as compared with 90% originally to a given dose (109). It was more DDT-tolerant, and contained more Fe than before selection (211). The HCN tolerance is associated with chromosome II, possibly a single locus (109). Tartar emetic tolerance was developed by pressure from that compound and proved to be nonspecific, extending to DDT and HCN also.

DDT tolerance developed at Hikone, Japan, after three years of antimalarial spraying (480). Strains from Fukuoka and Kochi were found to be naturally more DDT-tolerant than elsewhere (246). DDT pressure on adults of a laboratory strain at Madison, Wisconsin, raised the tolerance by 6–8 times in three years (108). Adult pressure at Cold Spring Harbor raised it 2–3 times in 20 generations (248); larval pressure scarcely increased it, but combined larval and adult pressure raised it 6–8 times in 26 generations (250). The natural tolerance extended to BHC, chlordane, parathion, DNOC, and nicotine (246). The induced DDT tolerance extended to BHC (479,497), DFDT (29), and other chlorinated hydrocarbons (497), but not to HCN (211), parathion, or pyrethrins (497).

DFDT tolerance was induced by 32 generations of DFDT pressure, but it still allowed 30% kill by doses that originally killed 70% (422). BHC tolerance involving an increase in LC_{50} of almost 4 times was obtained by 13 generations of BHC pressure, but little further increase occurred in 11 generations; the tolerance extended to DDT and Thanite (374). Nicotine tolerance was present in the Hikone strain (476) but not in the Madison strain (497).

DDT-tolerant strains developed by DDT pressure were larger and about 15% heavier than normal and took longer to develop (48). The females showed a 12% lower oxygen consumption, but the males were no different from the normal (47). By selecting individuals that pupated soonest, it was possible to decrease the average DDT tolerance of a strain; but it was not possible to increase it by selecting those that pupated the latest (224). One DDT-tolerant strain tended to pupate at the periphery of the larval medium, and a similar DDT-tolerant strain could be developed by selecting individuals that pupated at the periphery (456). Two Japanese DDT-tolerant strains and the Bayfordbury strain contained more Fe than two normal strains, and the inheritance of the increased Fe content paralleled that of the DDT tolerance (211). Individuals of the Madison strain do not dehydrochlorinate DDT (109).

The genetic origin of the induced DDT tolerance of most strains is evidently polygenic (48,108,248) with a certain degree of dominance (48,108). Each of the three chromosomes, X, II, and III, contributes at least one dominant and one recessive factor to the DDT tolerance of the Madison strain, and most of the dominant resistance in chromosome II is located in two regions close to the centromere on either side of it (109). But the DDT tolerance of the Hikone strain, which had been further increased by laboratory pressure on the larvae, was attributable mainly to a dominant allele of a gene located at crossover distance 66.7 on chromosome II (369). The BHC tolerance of this strain was also found to be dominant and referable to the same locus (479), and so was the DDT tolerance of the Fukuoka strain (478). The nicotine tolerance of the Hikone strain was mainly due to an incompletely dominant gene at map distance 49–50 near the centromere of chromosome II, enhanced by genes on other chromosomes (477). This gene on chromosome III may be the most important modifier of the DDT tolerance primarily controlled by the gene on chromosome II (375).

DDT pressure for 40 generations on two inbred strains failed to increase the DDT tolerance at all (248,327). Similar failure was encountered with a combination stock of five widely different strains, but each founded by one female. However, every one of six Minnesota strains, each originating from field collections of 10–50 flies, developed DDT tolerance under pressure (327). When two different DDT-tolerant strains were combined, the F_2 showed a very shallow dosage-mortality curve, indicating that different factors for tolerance had been contributed by each strain (249). During the laboratory induction of DDT tolerance in a number of strains, the dosage-mortality line first became shallower and finally became steeper (471); this is the criterion for reaching the maximum of tolerance (219), although in these cases it was no more than 8 times the normal. The DDT tolerance of the Madison strain showed no reversion in three years of relief from DDT (108).

Drosophila virilis. DDT resistance developed in 1952 also in this species at Hikone, Japan (480). Factors in chromosome II and chromosome V contributed equally to this DDT resistance. The strain was also nicotine-resistant, to which chromosome II contributed more than chromosome V. Chromosome II in *D. virilis* corresponds to chromosome III in *D. melanogaster*, and chromosome V to chromosome II; therefore the genes for DDT and nicotine resistance may be the same in the two species (376).

Fannia canicularis (little house fly). DDT resistance appeared in 1953 at Jaca, in northern Spain, after four to five years of treatments. On comparison with a normal laboratory strain at Basle, it showed only 5% knockdown in 4 hours' exposure to residues that normally caused 98% knockdown in 30 minutes (503).

Hylemyia antiqua (onion maggott). Dieldrin resistance was reported from several areas in Wisconsin in 1957, extending to other cyclodiene derivatives (125a); similar resistance has been reported from Michigan and southwestern Ontario.

Stomoxys calcitrans (stable fly). DDT resistance evidently developed in Sweden two years after the introduction of DDT (447a). Yet in Illinois the susceptibility of this species remained at the same LD_{50} level from 1945 to 1949 (65).

Muscina stabulans (false stable fly). Adults collected in 1951 from a California dairy where the house flies were resistant showed an even greater susceptibility than normal laboratory house flies (268). Similarly, *Siphona irritans* has failed to develop resistance even in barns exposed to DDT for ten years (258).

Musca sorbens. The question of resistance in this species remains unanswered. After an initial success the application of DDT and BHC to breeding sites in Morocco later encountered control failure (157). The pattern was repeated in Egypt, but when the adult *M. sorbens* were tested by topical application they showed a lower LD_{50} than *M. d. vicina* (386); however, this may be simply a consequence of their much smaller size (81).

Musca domestica. DDT resistance of the house fly first became apparent in 1946, and by 1948 many states were reporting its appearance. If these records are assembled with special reference to the year when DDT was introduced (Table I), it is evident that in most cases resistance appeared two years after introduction.

The subspecies *M. d. vicina* showed little resistance in tropical South America (390), in Ceylon it did not develop in the customary two years (71), and in Japan it finally appeared in 1953, eight years after the introduction of DDT (86). But DDT resistance in *M. d. domestica* was not reported from France until 1953 and Yugoslavia until 1955 (269) and Holland until 1956 (321).

TABLE I
Development of DDT Resistance in the House Fly

Year reported	Area	Years of DDT use	Ref.
1946	Sweden	2	498
	Denmark	2	239
1947	Italy	1	435
	Greece	1	288
	Alabama	3	208
	Mexico	2	208
1948	Sardinia	2	208
	New Zealand		201
	Florida	2	254
	Texas	2	254
	Georgia	2	208
	California	2	312
	New York	2	194
	New Jersey		21
	Illinois	3	65
	[a]Israel	2	446
	[a]Lebanon	2	514
	British Guiana	3	403
1949	West Germany		355
	England	2	196
	Corsica	1	231
	Uruguay		295
	Southern Brazil		439
	Arizona		440
	Oregon	5	430
	Eastern Canada	3	424
	[a]Egypt	1	514
1950	Switzerland		459
	East Germany		134
	USSR		60a
	Morocco	2	157
	Argentina	3	208
	New South Wales		208
	Western Canada	4	424

[a] *M. d. vicina* involved.

DDT resistance in the Indian *M. d. nebulo* house fly had not developed in Delhi State after four years of antimalarial house spraying (377,380). DDT pressure in the laboratory almost doubled the knockdown time in the F_2, but induced no further change in 24 generations (380). At this stage the mortality was 30–66% at doses completely lethal for the original strain, but it was not changed in 22 subsequent generations under pressure (381). However, this strain originated from a single female (442). Residual spraying with DDT at Aligarh has not induced DDT resistance in *nebulo* (245), but BHC resistance occurs in this form in Aligarh, Bombay, and Assam (414).

DFDT resistance appeared in West Germany in 1948, three years after the introduction of DFDT, evidently without changing the DDT susceptibility (330). On the other hand, DDT resistance extended to DFDT in Denmark (239). DDT resistance extended likewise to methoxychlor and DDD in Denmark (239), southern New York (21), and California (312). The methoxychlor resistance in northern New York and Florida was appreciably less than the DDT resistance (252,399) but prevented control (163), while in New Jersey it was sufficiently less to allow control with methoxychlor (191). The DDT resistance did not decrease the susceptibility to BHC, chlordane, toxaphene, or dieldrin (21,191,252,289,312).

Resistance to BHC, chlordane, and dieldrin was first reported in 1949 and appeared in many states in 1950. Table II shows that resistance usually developed one year after the introduction of BHC, chlordane, or dieldrin, and sometimes even in the same year.

Chlordane resistance in Sardinia extended to dieldrin, heptachlor, aldrin, and toxaphene and in less degree to BHC (72,294). BHC resistance extended to dieldrin in England (74,171), to chlordane and dieldrin in New Jersey (192), and in less degree to heptachlor, aldrin, dieledrin, and toxaphene in California (313). BHC-resistant *M. d. vicina* at Omdurman, Sudan, were more resistant to dieldrin than to γ-BHC (74), while the reverse was the case at Ilaro, Nigeria (81). It is difficult to find a BHC-treated area never exposed to DDT, but at Omdurman the BHC resistance was 60 times, while the DDT resistance was only 8 times, that of a normal strain; and a Uruguayan strain was 100 times BHC-resistant and 600 times dieldrin-resistant, but only 4 times DDT-resistant (74).

Laboratory selection of a normal strain by DDT or other chlorinated hydrocarbon insecticide has always notably increased the resistance, except in two instances: DDT pressure on a Palermo strain originating from one female (112) and BHC pressure on the Hawthorndale strain (51). Table III shows increases in resistance obtained by pressure on the NAIDM strain at Urbana, as measured by the multiplication of the LC_{50} on topical application in acetone (121).

TABLE II

Development of Resistance to BHC, Chlordane, and Dieldrin in the House Fly

Year reported	Area	Years of use	Ref.
	BHC resistance		
1949	California	1	313
	[a]Egypt	1	153
1950	Denmark	1	237
	New York	1	402
	Sweden	1	256
1951	Illinois	1	121
	Uruguay		363
1952	West Germany	2	355
	New Jersey		192
	[a]Lebanon	1	514
	[a]Nigeria	2	81
	Hong Kong	2	427
1953	England	3	171
	USSR (Ukraine)	4	60a
	Southern Brazil	1	403
	[a]Sudan	4	74
	[a]Ceylon	3	300
1954	[a]Japan	0	86
1957	[b]India		245, 414
	Chlordane resistance		
1949	Sardinia	1	208
1950	Italy	2	350
	Denmark	1	237
	Switzerland		459
	Florida	3	162
	Georgia	0	287
1951	Greece	2	184
	[a]Egypt	1	386
1952	New Jersey	2	192
	[a]Lebanon	1	514
1953	Uruguay		403

TABLE II (*Continued*)

Year reported	Area	Years of use	Ref.
	Dieldrin resistance		
1949	California	0	313
1950	Florida	1	162
	Georgia	0	287
	Arizona	1	440
1951	Illinois	1	121
1952	Switzerland	1	356
	New Jersey	0	192
1953	[a]Liberia	0	396
	[c]Nigeria	1	133
1957	[a]Kenya	1	305a
1958	[a]Japan	1	489a

[a] *M. d. vicina* involved.
[b] *M. d. nebula.*
[c] *M. d. curviforceps.*

TABLE III
Resistance Ratios for House Fly Strains Selected with Various Chlorinated Hydrocarbon Insecticides

Pressure	DDT	Methoxychlor	γ-BHC	Chlordane	Dieldrin
DDT	800	14	4	2	3
Methoxychlor	1	185	2	2	2
γ-BHC	4	1	425	15	21
Chlordane	2	2	22	1250	380
Dieldrin	8	4	20	840	20,500

Thus DDT pressure induced a certain amount of methoxychlor resistance, but not nearly so high as the DDT resistance. Methoxychlor pressure—on a strain that is initially tolerant of methoxychlor (23)—induced methoxychlor resistance without DDT resistance. Neither DDT nor methoxychlor induced resistance to γ-BHC, chlordane, or dieldrin, effecting only a slight increase in tolerance; a similar result was obtained at Ithaca (401). Conversely, pressure from γ-BHC, chlordane, or dieldrin did not induce resistance to DDT or methoxychlor, but only a slightly increased tolerance. This was also observed with BHC pressure on normal strains in New Hampshire (44) and California (308,314) and with chlordane pressure on an Italian strain at London (73). BHC pressure induced a certain amount of resistance to chlordane and dieldrin, while chlordane or dieldrin pressure induced a certain amount of BHC resistance; but the induced resistance was always the greatest to the particular insecticide that was the selecting agent. Further experiments showed that BHC resistance and dieldrin resistance could be induced much faster in strains already DDT-resistant than in normal strains (65, 121). Evidence that multivalent resistance may be produced by pressure from any one chlorinated hydrocarbon alone has been reported for strains in Holland (320) and Switzerland (459).

Resistance to Dilan, to which DDT-resistant flies were susceptible because they could not detoxify it, rapidly followed its introduction in Florida in 1950 (507). Dilan pressure on the NAIDM strain induced resistance both to Dilan and to DDT (60), and prolan pressure on the super-Pollard strain increased the prolan resistance 1000 times in 12 generations (314) and the DDT resistance even more (328). The DDT-resistant strain from Torre in Pietra, Italy, was already resistant also to prolan (509) and to DANP (72). Resistance to DMC-synergized DDT developed within a few generations in Italy (351) and at Savannah, Georgia (138,139). Resistance to the synergistic effect of 1,1-bis-(*p*-chlorophenyl)ethane upon DDT increased 100 times in 5 generations of laboratory selection (316). Pyrolan resistance developed quickly in Italy (351) but not in Switzerland (503). Laboratory selection with pyrolan on a Basle strain produced no resistance in 9 generations (504) but much pyrolan resistance later (503). Chloroacetic acid was one house fly poison which did not induce resistance to itself in 30 generations of selection pressure (40). *N*,*N*-dimethyl phenylcarbamate (S.17) was another, but it induced resistance to DDT, BHC, and chlordane and very high resistance to dieldrin and toxaphene (320).

Pyrethrin resistance has recently appeared in the field, on the Wasaw farm near Stockholm, Sweden, where synergized aerosols with piperonyl butoxide have been employed until ultimate control failure; the resistance to pyrethrins proved to be 10–14 times the normal (238a). Pyrethrin tolerance was increased only 6–10 times in 220 generations of pyrethrin pressure on a normal strain at Edgewood (93), but pyrenone tolerance was increased over 20 times in 30 generations of pyrenone pressure (121). Selection pressure of various chlorinated hydrocarbons on the NAIDM strain (121) or of parathion on the Berkeley strain (311) did not increase the pyrethrin resistance (121); the DDT-selected Orlando strain increased in pyrethrin tolerance at the same rate as DDT tolerance (251,508).

Resistance to organophosphorus insecticides appeared in South Zealand and other parts of Denmark in 1955, where parathion had been used since 1952, diazinon since 1953, and Resitox in 1955. The resistance to parathion was 2–3 times, to diazinon 3–15 times, and to Resitox 8–45 times the normal; indeed, on one farm in North Jutland, where only parathion had been used, the Resitox resistance was 500-fold after the first spraying (238). It was considered in Denmark that a 10-fold increase in resistance may be sufficient to invalidate residual sprays of organophosphorus compounds (238).

Malathion resistance was discovered in Florida in 1955 (283), and in 1956 the resistance in flies collected at Orlando and Tampa was, respectively, 37 and 23 times the normal, while they still remained susceptible to Dipterex (261a). It appeared also at Savannah, Georgia, where a physiological resistance 2.4 times the normal was reinforced by a behavioristic resistance of avoiding the malathion baits; again the resistance did not extend to Dipterex (247a).

Diazinon resistance developed in 1956 in the region of Latina, Italy, after 4 years of successful control with this compound; the field populations were 5–60 times as diazinon-resistant as normal (435a). Diazinon resistance also developed in canton Valais, Switzerland, with an even higher cross-resistance to parathion (503a). It finally appeared in New Jersey where diazinon sprays had been successful since 1953; in 1957 only 16% mortality was obtained by deposits that were normally completely lethal (193a).

Laboratory pressure experiments in Basle showed that parathion pressure induced resistance to diazinon and to malathion without decreasing the susceptibility to itself (158). Table IV shows the increases in resistance induced by laboratory selection with organophosphorus compounds in various separate experiments.

Increased diazinon tolerance has also been induced by larval pressure (326). The strains with induced diazinon tolerance also proved to be highly resistant to chlorinated hydrocarbons (320,503), and parathion pressure on a normal Riverside strain induced a 3000-fold resistance to DDT and dieldrin (311).

TABLE IV
Summary of Resistance Increases Induced by Laboratory Selection with Organophosphorus Compounds

Compound	Resistance (× normal)	Period (no. of generations)	Ref.
Parathion	9	10	401
	10	41	311
	20	149	311
Diazinon	10	13	320
	10	19	311
	5	31	311
Chlorthion	60	39	311
	10	19	311
Malathion	3	19	311
Dipterex	4	13	283
	8	12	283
Para-oxon	10	40	121
DFP	15	100	91
DDVP	8	8	140

Searchers for the physiological cause of DDT resistance early found that the tarsi were larger, darker, and more thick-skinned in the Arnas resistant flies than in a Basle strain (498); but this may be a normal characteristic of northern Sweden. A greater pulvillar width was found in the resistant Italian strain (498), but no greater cuticular thickness and tarsal size could be detected in the same Italian strain (39) or in DDT-resistant strains from California (309) and New York (unpublished data).

DDT-resistant strains were no different from the normal strains in the viability of the various stages (483). There was no difference in egg production or the percentage hatching (19,400). Despite an early report that DDT pressure increased the average size (24), later work found no difference in pupal or adult weights associated with DDT resistance (305,309,400,483). But it was observed that certain DDT-resistant strains in Illinois took 1–2 days longer from hatching to emergence (63), and selection of the individuals emerging latest in a strain slightly increased the average resistance, while selection of the early emergents decreased it (121). DDT-resistant strains in New York showed a 20–80% longer larval life than the normal NAIDM strain, and the individuals pupating latest within one of these four strains were about 10 times as DDT-resistant as those pupating earliest (400). But the DDT-resistant Bellflower strain had no longer larval life (309); and when several resistant and susceptible strains were compared, there was no consistent difference (19,368). Nevertheless, selection of late pupaters in the Super-Laton strain increased the larval period by 50% and the DDT resistance 7 times in 15 generations, but this resistance reverted in 38 further generations of selection (305).

DDT-resistant flies have been observed as being more sluggish (65,338), staying at floor level (254,347), or being relatively repelled from DDT-treated surfaces, presumably by irritation (254). These phenomena are sublethal effects on flies which are now resistant and not an intrinisic behavioral change; no such change has been observed in laboratory selection (347).

Lowered cuticle permeability is not the cause of DDT resistance. Resistant strains continued to show more resistance than susceptible ones when the DDT was injected into the haemolymph (39,72,312). Either no significant difference in the rate of absorption of DDT was found between susceptible and resistant strains (465,512), or there was no consistency in the differences when many strains were compared (390,395); nevertheless, certain DDT-resistant strains are known which absorb much less than the normal (236).

All DDT-resistant strains studied have been found to dehydrochlorinate this insecticide to DDE (308,390,391,395,465,509). On the other hand, susceptible strains produce very little DDE (308,390, 392,509) or in certain instances, such as the Beltsville and Multi-X strains, no detectable amounts at all (145,395,465). The dehydrochlorinating activity is especially high in the integument (218,462, 470), but it is present in other organs, including fat body and nerve (218,339). Dehydrochlorination of DDT *in vivo* is inhibited by piperonyl cyclonene (391) and DMC (394), which are effective synergists for DDT (392,468).

Homogenates of DDT-resistant flies dehydrochlorinate DDT to DDE *in vitro*, whereas those of susceptible strains do not (446,467). Glutathione is needed as an activator, but the conversion may sometimes proceed without it.* When the dehydrochlorinating activity was concentrated 120 times it showed 4 protein components (344), but on further concentration by 20,000 times it showed only 2 major components (287a). Termed DDT-dehydrochlorinase, it has an optimum pH of 7.4, requires activation by glutathione, acts only on *p,p′*-substituted DDT analogs, and produces only the corresponding ethylene derivative (466). It is inhibited by DMC and other chlorinated carbinol synergists (345). These inhibitors fail to synergize DDT with susceptible flies because they contain no enzyme to be inhibited. The DDT analogs most liable to be synergized by chlorinated carbinols are those most liable to enzymic dehydrochlorination (419). In resistant strains, the level of DDT-dehydrochlorinase increases during larval life and then is halved by the time of emergence; late pupaters and emergents contain more DDT-dehydrochlorinase (346).

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* Evidence for metabolism of DDT (as measured by bioassay of a chromatographic spot) has been obtained in homogenates of a susceptible strain, without added glutathione (418).

Seven DDT-resistant strains produced virtually nothing but DDE from DDT topically applied (393). No DDA is produced (18,308, 463). But there are several instances of the DDE produced falling considerably short of the DDT destroyed (18,145,391,392,465) and the short-fall has been called "metabolite X" (470). Evidence has been obtained for the eventual production and excretion of a water-soluble conjugate (473). Radioactive studies indicate that no DDT is metabolized as far as CO_2 (429).

Since dehydrochlorination stops at death (391,425,509), the suggestion had been made that the greater DDT-DDE conversion in resistant flies was due simply to their surviving longer than susceptible ones (90). Susceptible flies of one strain converted the same proportion of its small absorbed dose as did a resistant strain of its large dose (284). However, when exposed to equal doses that were just sublethal for the susceptible strain, resistant adults and larvae converted 3 times as much of the absorbed dose as susceptible adults and larvae (324).

Since resistant flies carry enough unconverted DDT to kill many susceptible flies(18,145,392,470), it has been suggested that the resistance lies in the nerve itself rather than in detoxification (405,487, 498). Certainly the nervous activities of resistant flies are less susceptible to DDT poisoning. Susceptible flies lose their proboscis reflex to sugar on tarsal application of DDT, but resistant flies do not (500). Susceptible flies may have their tarsal sensitivity to sugar increased by minute doses of DDT, but resistant flies remain unchanged (455).

Legs of DDT-poisoned susceptible flies continue trembling after amputation, while those of resistant flies cease their tremors (498). DDT applied to the thoracic ganglion induces leg tremors more frequently and persistently in susceptible flies than in DDT-resistant ones (405). Electrophysiological study of isolated sensory nerves showed that those of resistant flies can recover from DDT poisoning where those of susceptible flies cannot (487).

However, it is entirely likely that the greater resistance of the nerves of resistant flies may be due to their greater content of DDT-dehydrochlorinase (94,339). The concept of balance between rates of supply of DDT to vital centers and its detoxification can apply to the observations that (*1*) a rest period between exposures allows a greater aggregate dose to be withstood (216), (*2*) resistant flies can repeatedly recover from knockdown when that action ensures the exposure is sublethal (22,72,202), and (*3*) DDT-treated flies can be prostrated by cooling and recover again on warming (470). The last phenomenon is ultimately due to the temperature coefficient of absorption being less than that of DDT-DDE conversion; at low temperatures absorption outruns detoxification, and at high temperatures detoxification outruns absorption (324). Resistant flies, like normal ones, show lower mortalities when held at high temperatures, despite higher absorption; mere activity does not increase absorption (429).

No other enzymic differences have been found to be associated with resistance when a number of strains were compared. There was no consistently greater content of cholinesterase in DDT-resistant than in susceptible flies (150,406). A multiresistant Sardinian strain contained no more triosephosphate dehydrogenase than a normal Rome strain and was no less susceptible to chloroacetic acid (40). Succinic dehydrogenase preparations from a resistant and a normal strain were equally susceptible to the *in vitro* inhibition by DDT and related compounds (3). Although certain strains contained more than others (436), there was no consistently greater cytochrome oxidase in resistant than in susceptible flies (395). Resistant strains were not characterized by a greater rate of oxygen consumption than the normal (151,276,406,437); nor did they consistently contain more acid or alkaline phosphatase than the normal (2a).

Studies of a limited number of strains have revealed some suggestive differences in chemical content. Adults of the resistant Orlando-Beltsville strain contained about two-thirds more Cu than the normal

CSMA strain (423). Two resistant strains contained 10–30% more lipid than a susceptible strain, and the lipids were more unsaturated (501), the DDT resistance of normal strains could be increased by feeding or injecting fats (325,501). The cholesterol content of the nerve of two strains of DDT-resistant flies was 2–3 times that of a normal strain, and vital dyes penetrated into their epidermal cells with great difficulty (502). Nevertheless, no consistent differences between the chemical contents of resistant and susceptible strains could be detected by paper chromatography of fresh tissue extracts, although populations from different geographical sources did show consistent differences (310).

BHC-resistant strains metabolize γ-BHC and the other isomers at a faster rate than normal strains (54,55,371–373,464), and the rate is correlated with the resistance (373). Most BHC-resistant strains absorb less γ-BHC than the other isomers, there being a correlation between absorption rate and susceptibility (54,55,373), although an Illinois strain absorbed no less than the normal (464). γ-BHC is first dehydrochlorinated to pentachlorocyclohexene, by a mechanism not involving DDT-dehydrochlorinase (464), and no trichlorobenzene is produced (54). The pentachlorocyclohexene is subsequently broken down (464), finally being converted to water-soluble products which are excreted by the fly (55). Heptachlor and aldrin are metabolized to heptachlor epoxide and dieldrin, which are more toxic (388) and may be the effective toxins (219). Heptachlor-resistant flies, however, are killed by contact application of the epoxide, although they survive the similar application of heptachlor, which they metabolize to the epoxide (388). Dieldrin-resistant flies developed from a normal strain contain no demonstrable DDT-dehydrochlorinase (236).

Certain morphological abnormalities, particularly of the wings, have appeared in DDT-resistant strains. Black abdominal sclerites characterized the Illinois DDT-1 strain (271), and a broken costa was characteristic of the inbred strain 1R-1 derived from it (272). The Bellflower, Pollard, and Beltsville resistant strains showed a greater ratio of width to length of the second abdominal sternite than 4 susceptible strains (41). However, a further study of 9 strains found that the variations among strains were such that no significant correlation could be deduced between resistance and this character or 16 other morphological characters (457). Nevertheless, when 19 strains were ranked according to sternite width-length ratio and DDT resistance, the 9 lowest were all susceptible strains (348). A laboratory-selected DFP-resistant strain contained an exceptionally high proportion of abnormalities in the wing venation (91). Since the association of abnormality with DDT resistance does not hold good for all strains and all individuals, these characters must derive from other genes either linked or accidentally associated with resistance. The character "plexus" (reticulation of the posterior transverse vein), which was of frequent occurrence in a resistant strain from Latina, did not show Mendelian segregation in its inheritance, although homozygotes for it tended to be more DDT-resistant than heterozygotes (333,335).

DDT resistance is primarily of monofactorial origin. The gradual increase in resistance on selection of the NAIDM strain was originally considered to have indicated polygenic inheritance (65), the data from Californian selected strains were so interpreted (107), and the resistance of Italian and Sardinian strains was also considered to be polygenic (262). But study of the highly resistant Multi-X strain showed that it was not homogeneous, certain individuals having strong resistance superimposed on weak tolerance; this strong resistance was due to a single gene showing dominance, the weak tolerance being due to polygenic and environmental causes (306). This gene was found to be present also in wild Illinois populations; by repeated crossing with susceptibles and culling with a level that kills the susceptible homozygotes, females were obtained which always gave a 50:50 offspring ratio in a back-cross with susceptibles (273) Resistance was imparted only through this gene, ancillary genes merely serving to modify the resistance level of heterozygotes (271). The DDT resistance of one Danish strain from Tillitze displayed the segregation typical of a single recessive gene, but another strain from Nalsgard showed evidence for more than one gene being involved (25,237).

The knockdown resistance which characterized the Torre in Pietra strain (the original "*M. d. tiberina*"), after hybridization with the normal Roma strain, showed the segregation in the F_2 and back-cross from F_1 that indicated it was determined by a single recessive gene (197). The kill resistance of this strain showed no segregation (199). However, if the dosage-mortality data of the F_2 are more closely analyzed, it will be found that the central one-half of the F_2 fall between the same limits as the F_1, and one-quarter are below and one-quarter above (336). Flies of a DDT-resistant strain from Latina were characteristically kill-resistant, but many of them were not knockdown-resistant. The knockdown resistance derived from a single recessive gene, *kdr;* the heterozygotes were knockdown-susceptible, but a proportion of them were kill-resistant, the precise proportion depending on their sex and on the normal strain used in the experiment (333,335) The factors for brown body (*bwb*) and divergent wings (*dv*) are in the same linkage group as *kdr,* but removed from it by a large crossover distance (337).

BHC resistance may be due to many genes. Crosses between a BHC-resistant, DDT-susceptible Uruguay strain and a normal strain showed no detectable segregation of this character in the F_2 (82). The BHC resistance of a multiresistant Danish strain was similarly found to be polygenic with a tendency toward dominance (25,237). Chlordane and BHC resistance are genetically different from DDT resistance (25,73,262). Increase of DDT resistance by selection, or decrease of DDT resistance by reversion, has been obtained without essentially changing the BHC resistance; and DDT-resistant may be segregated from DDT-susceptible phenotypes while all individuals remain equally BHC-resistant (25). Repeated back-crossing of a multiresistant strain, still under BHC selection, with a susceptible strain eliminated the DDT resistance while maintaining the BHC resistance. Resistance to chlordane and dieldrin also remained inseparably associated with the BHC resistance (73).

Phormia regina (black blowfly). Adults collected in 1951 from a California dairy barn, where house flies were DDT-resistant, proved to be more susceptible to DDT and methoxychlor than a normal laboratory strain (308,314). Laboratory selection with γ-BHC for 30 generations did not increase the BHC resistance (328).

Phaenicia pallescens. Dieldrin resistance was not induced in this green-bottle fly by 6 generations of severe dieldrin pressure (137). The DDT susceptibility of *P. sericata* in DDT-treated barns was reported unchanged in 1953 (315).

Lucilia cuprina (sheep blowfly). Control failures with aldrin or dieldrin appeared on three properties in New South Wales. Laboratory tests with topical application on the adults and with immersion of the larvae showed that the dieldrin resistance was 10–100 times the normal (441a).

Callitroga hominivorax (screwworm). Resistance to phenothiazine was developed by 8 generations of larval pressure, so that larvae showed only 43% mortality to dosages that formerly caused 94–100% mortality. Larvae of this species normally show great variation in their susceptibility. No resistance was observed in the field (256). Adults of the secondary screwworm, *C. macellaria*, were still susceptible where the house flies had become resistant (308).

Chrysomyia putoria. BHC resistance of this latrine fly appeared in 1949 at Leopoldville, Belgian Congo, four months after BHC was introduced to treat the breeding places. Substitution of diazinon in 1953 led to 6-fold diazinon resistance in 1954 and 300-fold in 1955, involving some cross-resistance to malathion, chlorthion, EPN, and even pyrolan. Substitution of malathion in 1955 led to increase of malathion resistance and decrease of diazinon resistance (38).

Pulex irritans (human flea). DDT resistance was first reported from Peru in 1949 (434), two areas of Ecuador in 1950 (438), Greece in 1951 (184), and Pernambuco, Brazil in 1952 (438). It had also become evident by 1952 that 10% DDT dusts would no longer control *Pulex* in the Palestine refugee camps (209), although emulsions still could (174). All these reports suffer from lack of some standard test. The report from Ecuador included *Ceratophyllus londinensis* and *Polygenis* spp. as showing the DDT resistance (438).

Ctenocephalides canis and C. felis (dog and cat fleas). DDT resistance of *C. felis* was demonstrated in 1952 at Savannah, Georgia (247). DDT resistance of *C. canis* was reported in 1953 from French Guiana (147), British Guiana, and Colombia (403). Dog and cat fleas subsequently became sufficiently resistant to chlorinated hydrocarbons at many points in the United States that organophosphorus compounds were substituted (458). BHC resistance developed in *C. felis* at Hong Kong in 1957 (427a).

Xenopsylla cheopis (oriental rat flea). DDT resistance was claimed in two areas of Ecuador in 1950 (438), and a possibly slight increase of tolerance in Georgia in 1951 (208). DDT pressure on adults at Savannah increased the tolerance in 3 generations (to 32% mortality from doses that formerly killed 65%) but not in the subsequent 4 generations (247). Similar selection at London doubled the LC_{50} in 4 generations (444). But by 1956 there was no evidence of DDT resistance of *Xenopsylla* in the United States.

Boophilus decoloratus. Arsenic resistance of this cattle tick first appeared in 1938 near East London, Cape Province, and spread 80 miles along the coast by 1940, extending north to Zululand in 1945 (492,493). In 1944 the resistant ticks showed 63% survival to doses of sodium arsenite which allowed only 1% survival of a normal strain (370).

BHC resistance appeared in 1948, 18 months after BHC was introduced in the East London district. Only 4% control was obtained in γ-BHC dips that killed 100% from a normal source (495). It then appeared in the Bathurst district, which 10 years previously had been the second to report arsenic resistance. These ticks were resistant to toxaphene (494) and dieldrin (142), but had only a slight tolerance for DDT; the arsenic resistance persisted, although sodium arsenite had not been used for four years. However, BHC-resistant ticks are known in the Pretoria district, Transvaal, that are arsenic-susceptible (33).

DDT resistance appeared in 1956 on a farm in the East London. district (490). The LC_{50} was 13 times that of the normal arsenic-BHC-resistant tick of that area.

Boophilus microplus. Arsenic resistance appeared in Argentina about 1935 (143) and also developed in Colombia, in Jamaica, and in Sao Paulo (14) and Rio Grande do Sul (149), Brazil. It was present in central and northern Queensland by 1937 (212) and reached southern Queensland (the Beaudesert area) in 1949 (367) and New South Wales in 1954 (213,263).

Resistance to BHC appeared in the Rockhampton district of central Queensland in 1950, 18 months after its introduction, with an LC_{50} of 185 times the normal; it appeared in the Beaudesert area in 1952, two years after BHC was first used there. The Rockhampton ticks were resistant also to toxaphene and dieldrin, and somewhat more tolerant of chlordane and DDT (212); the Beaudesert ticks were resistant to toxaphene, dieldrin, and aldrin, while remaining susceptible to DDT and fairly arsenic-susceptible (367). Dieldrin resistance also developed near Brisbane (467b). BHC resistance appeared in 1952 in Rio Grande do Sul, Brazil, and extended to toxaphene and dieldrin (103a,149).

DDT resistance appeared in several localities near Rockhampton in 1954–55 (262a), with LC_{50} levels 22 times greater than normal (467a). By 1956 the cattle ticks in Queensland showed the following resistance ratios: BHC, 200 times; dieldrin, 2000 times; and DDT, 20 times; however, they did not dehydrochlorinate DDT to DDE (431).

Boophilus annulatus. Toxaphene resistance was reported by the Oklahoma Agricultural Experiment Station in 1954 (257).

Rhipicephalus sanguineus (brown dog tick). Chlordane resistance developed between 1953 and 1955 in New Jersey, where applications had commenced in 1949. These ticks were immune to exposure to 10% chlordane emulsion for two days, but were highly susceptible to γ-BHC (193). Chlordane resistance developed at various points in the United States in 1956 (458).

Argas columbarum. Resistance of this species of fowl tick to DDT, BHC, and dieldrin was reported from Junja, Colombia, in 1953 (403).

Metatetranychus ulmi (European red mite). Parathion resistance appeared in an orchard at New Paltz, New York, in 1952; when submitted to twice the concentration that controlled 99.9% in other orchards in the state, this population showed only 57% control. The resistance extended to malathion, but EPN and Systox were still practically effective (274). Resistance to parathion appeared in the Wenatchee Valley, Washington, in 1950, and in the Yakima Valley in 1951, when parathion had been used since 1947 (361). Tests at Yakima in 1952 obtained no control with 4 times the dosage that controlled in 1948. The resistance also was shown by *Tetranychus pacificus* and *T. mcdanieli.* Here malathion could still control, as well as EPN, Systox, Aramite, DMC, and K-6451 (362). Resistance of spider mites to organophosphorus compounds was reported for California in 1957, as well as for Mississippi and South Carolina where sulfur insecticides were also involved (153a).

Resistance to parathion and malathion was reported from British Columbia in 1956 (127a). It was also present in California in 1957 and extended to EPN, demeton, and diazinon, but scarcely yet to Trithion and Guthion. Although resistant to Ovex, Genite, and Chloroparacide, the red mites were still susceptible to sulfenone, chlorobenzilate, and Aramite (26a).

Tetranychus bimaculatus (two-spotted spider mite). Selenium tolerance was noted in 1937 in mites bred on roses, which when tested on cotton cloth showed only 2% mortality to doses of Selocide (ammonium potassium selenosulfide) that killed 100% of a culture from snapdragon (103). A gradual increase in Se tolerance was noted as the red form of the mite was cultured on roses (359); an increasing proportion of green forms was produced, which showed only 46% mortality to doses which killed 98% of the red form (360). Selocide soon became ineffective in sprays in New York (382), and Se resistance was present throughout the eastern United States in 1943 (409); however, some strains were reported in 1952 to be still susceptible to sodium selenate applied through the soil (449). It was noted that Mo added as ammonium molybdate to the soil would counteract the systemic effects of Se (154). Rotenone pressure applied for three months to a strain in Wooster, Ohio, decreased the mortality induced by a given rotenone treatment from about 70% to approximately 50% (360).

Parathion resistance first appeared on greenhouse roses in 1949 at Cromwell, Connecticut, Cranbury, New Jersey, and Bloomsburg and North Wales, Pennsylvania, one year after parathion succeeded TEPP as an acaricide (154,450). Control failures with TEPP had appeared but had been overcome in two of these greenhouses in 1947 (450). The resistant strain showed only 15% mortality to concentrations that normally killed 100% (154). In 1949 the parathion resistance spread to other greenhouses and into New York State (382), and by 1950 it affected a large percentage of the rose growers in the eastern United States. The Cromwell strain lost its resistance after four months of rearing on beans (154), but other strains retained it for ten months on beans and other host plants (449).

Resistance to parathion and other organophosphorus compounds developed in two-spotted spider mites on greenhouse roses in southern California about 1949, and was followed by Aramite resistance in 1952 and chlorobenzilate resistance in 1955; in 1956 azobenzene was found completely effective as an additive to the two previous compounds (231a). Parathion resistance appeared in *T. bimaculatus* in apple orchards of Elgin district, western Cape Province, South Africa, in 1957 (491).

Parathion resistance extends in slight degree to other organic phosphates, but TEPP, Sultotepp, and Systox can still exert control (449,450). By use of the associated malathion resistance as a criterion, resistance was found to be inherited as a single dominant Mendelian factor; the males are haploid (472). No morphological differences were observed between the resistant and nonresistant strains (154,472).

Tetranychus urticae. Parathion resistance appeared in this European species on carnations at Stuttgart, Germany, in 1950, and maintained itself even in absence of parathion (126). It also appeared on carnations at Nice, France, extending to all other organophosphorus compounds tested, but not to chlorobenzilate, Aramite, or Ovex; it was stable on removal from insecticidal pressure for three years. A normal strain of *T. urticae* at Basle could become resistant to chlorobenzilate and diazinon as fast as to parathion when under pressure of these particular compounds. The parathion-resistant and chlorobenzilate-resistant strains developed faster than the normal strain at all temperatures at 90% R.H.; at the lower temperatures (16–22°C.) and humidities (60–30%) they developed slower than the normal (156a).

Metatetranychus citri. Parathion resistance of 7,000 times the normal was reported from Corona, California, in 1957, the resistance trithion was even higher, while it was quite high against diazinon and demeton, and quite low against malathion (231b).

Tetranychus tumidus. Parathion tolerance was first observed in this cotton spider mite in 1952, when contamination of a Texas laboratory was found to have raised the LD_{90} of a colony by 5 times. Systematic selection with parathion for three months raised the LD_{50} by about 20 times, but little further increase was induced by a subsequent four months of pressure (229).

Vasates schlechtendali (apple rust mite). Parathion resistance developed in British Columbia in orchards that had received 3 annual sprays for 2 years before 1955 (346a).

Scutigerella immaculata (garden centipede). DDT tolerance appeared in the garden centipede at Terre Haute, Indiana, in 1953; it became necessary to raise the dosage by 6 times to obtain the former degree of control (16).

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SUBJECT INDEX

A

Accedes fuscipunctella, 211
Accedes ganomella, 211
Accedes pallescentella, 211
Accedes semifulvella, 211
Aceria tulipae, 168
Activated charcoal seed coating, 161–63
Aedes aegypti, *369*
Aedes canadensis, 371
Aedes communis, 371
Aedes dorsalis, 370
Aedes fitchii, 371
Aedes nigromaculis, 370
Aedes punctor, 371
Aedes sollicitans, 370
Aedes taeniorhynchus, 370
Aedes vexans, 371
Aeolus mellilus, 139
Aeolus sp., 139
Agonoderus comma, 138
Agriotes mancus, 139
Alabama argillacea, 363
Aldrin, 141, 155, 247
Alfalfa, dalapon residues in, 201
  Di-Syston residues in, 174
  Thimet residues, in, 174
N-*n*-Alkylethylenethiourea compounds, 128
Alternaria oleracea, 119, 127
Alternaria tenuis, 116
Amino acids, 218, 220
Ammonium molybdate, 396
Amydria vastella, 210, 211
Anarsia lineatella, 363
Anchastus sp., 139
Anopheles albimanus, 376
Anopheles atroparvus, 375
Anopheles culicifacies, 375
Anopheles darlingi, 376
Anopheles fluviatilis, 375
Anopheles funestus, 373
Anopheles gambiae, 353, 372
Anopheles maculipennis, 375
Anopheles pseudopunctipennis, 376
Anophelcs quadrimaculatus, 316, 371
Anopheles sacharovi, 373
Anopheles stephensi, 374
Anopheles subpictus, 375
Anopheles sundaicus, 374
Anopheles superpictus, 375
Anthonomus grandis, 366
Anthrenocerus australis, 211
Anthrenus fuscus, 211
Anthrenus museorum, 211
Anthrenus pimpinellae, 211
Anthrenus scrophulariae, 211
Anthrenus verbasci, 211
Anthrenus vorax, 211
Aoniediella aurantii, 361
Aphis gossypii, 360
Application of pesticides, 1
Aramite, 395, 397
Argas columbarum, 395
Argyrotaenia velutinana, 363
ω-(Aryloxy)alkanecarboxylic acid amides, 298
ω-(Aryloxy)alkanecarboxylic acid nitriles, 298
Aspergillus niger, 119, 120, 122, 123, 127, 129
Aspidiotus perniciosus, 361
Atomization, effect of additives, 54
  of liquids, 2, 76
  principles of, 38
Atrolactic acid, 274
Attagenus elongatulus, 211
Attagenus gloriosae, 211
Attagenus lobatus, 211
Attagenus nigripes, 211
Attagenus pellio, 211
Attagenus piceus, 211
Attagenus schäfferi, 211
Auxins, 264
Auxocontacts, 313

Avena curvature test, 266
Azobacter agile, 118
Azobenzene, 396

B

B-complex vitamins, 214
Beet armyworm, 167
Behavioristic resistance, 376, 387
Beneficial insects, 148
Benzene hexachloride, 140, 247, 309, 390
BHC, metabolism of, 390
Biological control of insects, 240
1,1-Bis-(*p*-chlorophenyl)-2,2-dimethylpropane, 347, 348
1,1-Bis-(*p*-chlorophenyl)-ethane, 384
1,1-Bis-(*p*-methylphenyl)-2,2-dimethylpropane, 348
1,1-Bis-(*p*-methoxyphenyl)-2,2-dimethylpropane, 348
Blapstinus sp., 167
Blatta orientalis, 317, 357
Blattella germanica, 356
Boophilus annulatus, 395
Boophilus decoloratus, 393
Boophilus microplus, 394
Bordeaux mixture, 118, 119
Brevicoryne brassicae, 148
2-Bromo-3-nitrobenzoic acid, 291
Bucculatrix thurberiella, 167

C

Cabbage aphid, 148
Cabbage maggot, 137
Cadmium, uptake by spores, 123
Calandra granaria, 317, 335, 348
Calcium thioglycollate, 248
Calliphora vomitoria, 336
Callitroga hominivorax, 392
Callitroga macellaria, 393
Captan, 142, 164
Carbowax 6000, 160
S-(β-Carboxyisopropyl)dimethyldithiocarbamate, 279
S-Carboxymethyl diethyldithiocarbamate, 279
S-Carboxymethyl dimethyldithiocarbamate, 279
O-Carboxymethyl dimethylthiocarbamate, 279
S-Carboxymethyl dimethylthiocarbamate, 279
Carnitine, 219
Carpet beetles, 209
Carpocapsa pomonella, 363
Carrot rust fly, 138
Cell membrane of fungus spores, 129
Cephalosporium acremonium, 119, 125, 126
Ceratophyllus londinensis, 393
Ceresan, 164
Ceresan M, 142
Cerium, uptake by spores, 112, 123
Chemically modified wool, 247
Chitin, 239
  digestion by insects, 237
Chitinase, 239
Chloranil, 142
Chlordane, 247, 309, 381
Chloride determination, 193
Chloroacetic acid, 384, 389
Chlorobenzilate, 397
2-Chloro-1-naphthoic acid, 269
2-Chlorophenoxyacetic acid, 280
4-Chlorophenoxyacetic acid, 280
2-Chlorophenoxymethylphosphinic acid, 272
4-Chloro-*o*-toloxyacetic acid, 184
Chlorparacide, 395
Cholinesterase, 389
Cholinesterase assay of insecticides, 172
Chromaphis juglandicola, 360
Chromium fluoride, 243
Chrysomyia putoria, 393
Cimex hemipterus, 353, 359
Cimex lectularius, 359
Cimex rotundatus, 360
Circulifer tenellus, 166
Cirsium arvense, 295
Claviceps purpurea, 110
Clivina impressifrons, 138
Clothes moths, 209
Cl^{36} radiotracer, 194

Coccus pseudomagnoliarum, 361
Cochliobolus miyabeanus, 117
Compound 12008, 160
Compound 12009, 160
Configuration of DDT analogues, 330
Conoderus falli, 365
Contactophores, 313
Copper, uptake by spores, 110
Copper sulfate, 110
Cotton, metabolism of systemic insecticides in, 152
Cottonseed, dalapon residues in, 203
  Thimet residues in, 175
Ctenicera aeripennis, 139
Ctenocephalides canis, 393
Ctenocephalides felis, 393
Culex fatigans, 367
Culex molestus, 369
Culex pipiens, 368
Culex quinquefasciatus, 336
Culex tarsalis, 369
Cu^{64} radiotracer, 117
Curly top virus, 166
Δ^1-Cyclohexenyleneacetic acid, 273
Cyclohexylacetic acid, 273
Cysteine desulfhydrase, 232
Cytochrome oxidase, 389

D

Dacus dorsalis, 376
Dalapon, 185, 191, 198
Dalopius pallidus, 139
Darkling ground beetles, 167
DDA, 388
DDD, 327
DDE, 387
DDT, metabolism of, 356, 357, 387
  for mothproofing, 246, 254
DDT analogues, structure and activity of, 307
  tetra-substituted, 333, 337
DDT-dehydrochlorinase, 388
DDT isomers, 318, 323, 325, 332, 335
DDVP, 386
Deltochilum gibbosum, 211
Dermestes frischii, 210
Dermestes maculatus, 210
Dermestid beetles, 210
Detoxication in insects, 233
DFDT, 313
DFP, 386, 391
Diabrotica undecimpunctata, 138
1,6-Diamino-2,2-difluorohexane, 243
1,1-Dianisyl-1,2-dimethylpropane, 347
Dianisyl neopentane, 384
  resistance to, 357
Diarthrothrips coffeae, 358
Diazinon, 143, 385
2,4-Dibromo-6-fluorophenoxyacetic acid, 283
1,1-Di-*p*-bromophenyl-2,2-dimethylpropane, 347
Dichlone, 126, 142
2,4-Dichloro-6-anilino-*s*-triazine, 127
2,3-Dichlorobenzoic acid, 291
2,5-Dichlorobenzoic acid, 291
2,6-Dichlorobenzoic acid, 291
2,2-Dichloro-1,1-bis-(*p*-chlorophenyl)-ethylene, 325
5,7-Dichlorocoumaran-3-one, 282
2,2-Dichloro-1,1-diphenylethane, 313, 327
2,4-Dichloro-6-fluorophenoxyacetic acid, 283
2,3-Dichloro-1,4-naphthoquinone, 126
2,4-Dichlorophenoxyacetic acid, 184, 264, 280
2,6-Dichlorophenoxyacetic acid, 280
3,4-Dichlorophenoxyacetic acid, 280
3,5-Dichlorophenoxyacetic acid, 281
γ-(2,4-Dichlorophenoxy)butyric acid, 297
α-(2,4-Dichlorophenoxy)propionic acid, 271
N-2,4-Dichlorophenylglycine, 274
2,4-Dichlorophenylthioacetic acid, 274
2,2-Dichloropropionate, hydrolysis of, 191
2,2-Dichloropropionic acid, 185, 191, 198
  Cl^{36}-labeled, 195
  solvents for, 192
Dieldrin, 141, 155

Dieldrin (*Continued*)
  for mothproofing, 247
  resistance to, 360
O,O-Diethyl *S*-ethyl-2-mercaptoethyl phosphorothiolate, 151
O,O-Diethyl *O*-ethyl-2-mercaptoethyl phosphorothionate, 151
O,O-Diethyl *O*-ethyl-2-mercaptoethyl phosphate, 151
O,O-Diethyl *O*-ethyl-2-sulfinylethyl phosphate, 151
O,O-Diethyl *S*-ethyl-2-sulfinylethyl phosphorodithioate, 152
O,O-Diethyl *S*-ethyl-2-sulfinylethyl phosphorothiolate, 151, 152
O,O-Diethyl *O*-ethyl-2-sulfinylethyl phosphorothionate, 151
O,O-Diethyl *S*-ethyl-2-sulfonylethyl phosphorodithioate, 152
O,O-Diethyl *S*-ethyl-2-sulfonylethyl phosphorothiolate, 151, 152
O,O-Diethyl O-ethyl-2-sulfonylethyl phosphorothionate, 151
O,O-Diethyl *S*-isopropylmercaptomethyl phosphorodithioate, 160
O,O-Diethyl *S*-*n*-propylmercaptomethyl phosphorodithioate, 160
Digestion of wool, 207
1,2-Dihydro-1-naphthoic acid, 277
1,4-Dihydro-1-naphthoic acid, 277
3,4-Dihydro-1-naphthoic acid, 276
Dilan, 384
Dimefox, 157
2,3-Dimethylbenzoic acid, 291
2,6-Dimethylbenzoic acid, 291
2,2-Dimethyl-1,1-bis-(*p*-fluorophenyl)-propane, 347
O,O-Dimethyl *O*-2-carbomethoxy-1-methyl vinyl phosphate, 151
3,5-Dimethylphenoxyacetic acid, 286
N,N-Dimethyl phenylcarbamate, 384
2,4-Dinitro-α-naphthol, 241
Dipole moments of DDT analogues, 338
Dipterex, 385
Disulfide reductases, 229
Di-Syston, 153
  effect on germination, 165
  metabolism of, 152, 173, 177
  P^{32}-labeled, 153
Dithiocarbamates, 127
DMC, 388, 395
Dosage-response curves for fungicides, 129
Drop size in sprays, 73
Drosophila melanogaster, 316, 333, 334, 354, 376
Drosophila virilis, 378
2,4-D-type herbicides, 263
2,4-DB herbicide, 297

E

Earias insulana, 365
Eberthella typhosa, 118
ED_{50} values for fungicides, 120
Eleodes spp., 138
Elodea canadensis, 117
Endolytic systemic insecticides, 150
Endometatoxic systemic insecticides, 150
Endrin, 141, 247
Endrosis lactella, 210
Epichlorhydrin, 249
Epilachna varivestris, 366
Epinasty test, 268
Epitrix cucumeris, 366
EPN, 395
Erythroneura elegantula, 360
Erythroneura lawsoniana, 360
Erythroneura variabilis, 360
Escherichia coli, 118
3-Ethyl-5-methylphenoxyacetic acid, 286
Ethyl *p*-nitrophenyl phenylphosphonothionate (EPN), 143
Eulan(s), 243
Eulan CN, 244, 251
Excretory products of insects, 230

F

Fabric pests, 209, 210
False wireworms, 138
Fannia canicularis, 379
Ferguson principle, 128
Fibroin, 238

Flea beetles, 139
Fluorene-9-carboxylic acid, 277
Fluorides, 250
  for mothproofing, 243
2-Fluoroethyl alcohol, 150
Formaldehyde, 247
Fungi, 107
Fungicides, comparative efficacy of, 131
Fungitoxicity, 107
Fusarium decemcellulare, 119
Fusobacterium, *118*

G

Galleria, 224
Gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane, 309
Geiger-Müller counter, 190, 194
Genite, 394
Germination, effect of insecticides on, 144
Glomerella cingulata, 118, 119
Glyodin base, 126
Glyoxal, 249
Glyptotendipes paripes, 367
Granular insecticides, 168
Grapes, dalapon residues in, 198
Growth-regulating activity, assessment of, 266
Guthion, 155, 394

H

Heliothis armigera, 363
Helminthosporium sativum, 127
Heptachlor, 141
2-Heptadecyl-2-imidazoline, 124
  C^{14}-labeled, 126
Herbicides, 263
Hercules 528, 155
Hofmannophila pseudospretella, 211
House fly, insecticide resistance in, 379
Hydrogen cyanide, resistance to, 361
Hydrogen ion concentration of insect gut, 225
Hydrogen sulfide as fungicide, 124
Hylemya antiqua, 137, 379
Hylemya brassicae, 137
Hylemya cilicrura, 137
Hylemya floralis, 137

I

Impact nozzles, 16
Indole-3-acetaldehyde, 271
Indole-3-acetamide, 270
Indole-3-acetic acid, 263
Indole-3-acetonitrile, 264, 270
Indole-3-ethanol, 271
α-(Indole-3)isobutyric acid, 277
Indole-3-methanesulfonic acid, 271
α-(Indole-3)propionic acid, 287
Innate toxicity of fungicides, 119
Insecticide-fertilizer mixtures, 169
Insecticide residues, 171
Insecticide resistance, 351
Isodrin, 141
α-Isopropylphenylacetic acid, 278
Isotope dilution for residue analysis, 183

K

Keratin, 208

L

Lanoc CN, 244
Lanthionine, 224
Laphygma exigua, 167
Lauseto-neu, resistance to, 357
LD_{50} values of fungicides, 108
Leptinotarsa decemlineata, 366
Leptocerca hirtula, 376
Leptohylemyia coarctata, 139
Life cycles of fabric pests, 213
Lime-sulfur, resistance to, 361
Limonius californicus, 139
Limonius canus, 139
Limonius clypeatus, 139
Lindane, 140, 185
  absorption and translocation of, 155
Lipoid solubility of DDT analogues, 314
Lucilia cuprina, 392
Lygus hesperus, 358

M

Macrocentrus ancylivorus, 367
Malathion, 155, 385
Maleic hydrazide, 265
Mandelic acid, 274
Mass median diameter, 75
MCBP herbicide, 297
Melanotus communis, 139
Melanotus oregonesis, 139
Melophagus ovinus, 331, 333, 334
Mercury, uptake by spores, 112, 120
Metabolite X, 388
Meta-Systox, 151
Metatetranychus ulmi, 395
Methoxychlor, 247
  resistance to, 357
Methyl cellulose, 161
2-Methyl-4-chlorophenoxyacetic acid, 264
γ-(2-Methyl-4-chlorophenoxy)butyric acid, 297
α-Methylenephenylacetic acid, 278
9-Methylfluorene-9-carboxylic acid, 277
Microorganisms in insect gut, 237
Mitin FF, 245, 252
Molecular models, of 2,4-D compounds, 285
  of DDT analogues, 317, 327
Molecular weights of DDT analogues, 314
Monolinia fructicola, 115, 116, 118, 122, 126, 128
Monopis corcicapitella, 211
Monopis dicycla, 211
Monopis ethelella, 211
Monopis ferruginella, 211
Monopis monachella, 211
Monopis pseudagyrta, 211
Monopis rusticella, 211
Monopis trimaculella, 211
Monopis weaverella, 211
Mosquitoes, insecticide resistance in, 367
Mothproofing, 207
Mothproofing agents, 240
  action of, 250
Musca domestica, 317, 335, 337, 379
Musca domestica nebulo, 380
Musca domestica vicina, 379
Musca, sorbens, 379
Muscina stabulans, 379
Mutations in insects, 352
Myrothecium verrucaria, 119, 126
Myzus cerasi, 361
Myzus persicae, 159, 360

N

1-Naphthoic acid, 276
2-Naphthoxyacetic acid, 264, 275
α-(2-Naphthoxy) butyric acid, 275
α-(2-Naphthoxy)isobutyric acid, 275
α-(1-Naphthoxy)propionic acid, 268
α-(2-Naphthoxy)propionic acid, 275, 287
1-Naphthylacetic acid, 263
1-Naphthylmethanearsonic acid, 272
2-Naphthylmethanearsonic acid, 272
1-Naphthylmethanephosphonic acid, 272
1-Naphthylmethanephosphonous acid, 272
1-Naphthylmethanesulfonic acid, 271
Aci-1-naphthylnitromethane, 272
Nemapogon granella, 210
Nematodes, 139
Neurospora sitophila, 119, 122, 126, 127, 129, 130
Nicotine, 154
Nutrition of fabric pests, 214

O

1,2,4,5,6,7,10,10-Octachloro-4,7-methano-4,7,8,9-tetrahydroindene, 309
Oligodynamic action of fungicides, 121
Oncopeltus fasciatus, 358
Onion maggot, 137
Onychiurus sp., 138
Optical isomers of 2,4-D compounds, 287
Ovex, 397
β-oxidation of growth regulators, 292, 300

P

P^{32} radiotracers of systemic insectisides, 152, 153, 157, 162, 176, 177
P^{32} in study of fungitoxicity, 122, 127
Papain, 224
Parathion, 141
Pea curvature test, 266
Pediculus humanus capitis, 357
Pediculus humanus corporis, 357
Penicillin, 128
Pentachlorocyclohexene, 390
Pentachlorophenol, 241, 245
Pentachlorophenoxyacetic acid, 286
Pepsin, 224
Periplaneta americana, 224, 357
Peronospora viticola, 110
Perthane, 247
Phaenicia pallescens, 392
Phaenicia sericata, 392
Phenoxyacetic acid, 280
ω-Phenoxyalkanecarboxylic acids, 295
Phenylacetic acid, 274
α-Phenylisobutyric acid, 274
α-Phenylpropionic acid, 274, 278
Phereoeca allutella, 211
Phereoeca uterella, 211
Phereoeca walsinghami, 211
Phlebotomus pappatasii, 367
Phormia regina, 392
Phosdrin, 150
Phosphatase, 389
Phyllotreta spp., 139
Physical control of insects, 240
Phytophthora infestans, 110
Phytotoxicity of systemic insecticides, 156
Pieris rapae, 224, 363
Piperonyl butoxide, 384
Plant growth regulators, 263
Plasmopora viticola, 116
Plutella maculipennis, 365
Potassium bifluoride, 243
Potassium borofluoroacetate, 243
Potentiometer, 193
Praecedes thecophora, 211
Pressure nozzles, 12, 78
α-Propylidenephenylacetic acid, 278
Proteolytic enzymes of insect gut, 225
Protoparce quinquemaculata, 363
Psila rosae, 138
Psorophora confinnis, 371
Psorophora discolor, 371
Psychoda alternata, 367
Puccinia graminis, 110
Pulex irritans, 393
Pyrausta nubilalis, 365
Pyrethrins, 384
Pythium spp., 164
Pythium ultimum, 143

Q

Quassia, 155
8-Quinolinol, 127

R

Radioactive counting, probable error of, 190
Radioisotopes in study of fungicides, 119
Radiotracers in study of fungitoxicity, 129
Receptor sites in spores, 120
Redox potentials, 227
Reducing conditions in insect gut, 227
Repellents, 240
Residue analysis, 172, 183
Resistance to insecticides, 351
Resitox, 385
Rhagoletis completa, 376
Rhipicephalus sanguineus, 395
Rhizopus nigricans, 119, 125
Rhizopus suinus, 263
Rotary atomizers, 28, 84
Rotatability of DDT analogues, 331
Rotenone, 155, 396

S

S^{35} radiotracers, 127, 184
Saccharomyces cerevisiae, 119
Saissetia oleae, 361
Salmonella pullorum, 118
Sapaphis plantaginis, 361
Sapaphis pyri, 361
Schradan, 160

Scirtothrips citri, 358
Scleroplasta liberiella, 211
Scolytus multistriatus, 367
Scutigerella immaculata, 397
Seed Collembola, 138
Seed-corn maggot, 137
Seed germination, 164
Seed treatment, 135, 146
  with systemic insecticides, 147, 158
Selective insecticides, 148
Selenium, 395
Selocide, 394
Sericin, 238
Shigella dysenteriae, 118
Silk, digestion by insects, 237
Silver, uptake by spores, 112, 120
Sitophilus granarius, 367
Sodium antimony fluoride, 243
Sodium fluoroacetate, 150
Soil treatment, 135
Southern corn rootworm, 138
Spores, permeability of, 128
Spotted alfalfa aphid, 148
Spraying, 4
Sprays, deposition of, 96
Sterols, 218
Stomoxys calcitrans, 379
Strobane, 247
Structure-activity relationships of plant growth regulators, 269
Sulfotepp, 396
Sulfur as a fungicide, 124
Surface mean diameter, 75
Swirl spray nozzles, 18
Systemic insecticides, 135
  metabolism of, 150
Systox, 151, 395
  metabolism of, 151

T

Taeniothrips simplex, 358
Tartar emetic, 358
Tenaga inquisitrix, 211
TEPP, 396
1,2,2,2-Tetrachloro-1,1-bis-(*p*-chlorophenyl)-ethane, 327
2,3,4,5-Tetrachlorophenoxyacetic acid, 286
2,3,4,6-Tetrachlorophenoxyacetic acid, 286
2,3,5,6-Tetrachlorophenoxyacetic acid, 286
1,1,4,4-Tetra-(*p*-chlorophenyl)-2,2,3,3-tetrachlorobutane, 316
1,2,3,4-Tetrahydro-1-naphthoic acid, 276, 277
cis-1,2,3,4-Tetrahydronaphthylidene-1-acetic acid, 279
trans-1,2,3,4-Tetrahydronaphthylidene-1-acetic acid, 279
Tetram, 160
Tetranychus atlanticus, 149
Tetranychus bimaculatus, 395
Tetranychus citri, 397
Tetranychus tumidus, 397
Tetranychus urticae, 396
Therioaphis maculata, 148, 160, 165
Thimet, 153
  effect on germination, 164
  metabolism of, 153, 173, 176
Thiram, 142
Three-point contact of growth regulators, 289
Thrips tabaci, 358
Tiefenwirkung, 154
Tilletia tritici, 111, 113, 114
Tinea columbariella, 211
Tinea fuscipunctella, 211
Tinea metonella, 211
Tinea pellionella, 211
Tineola, internal morphology of, 222
Tineola bisselliella, 210
Tineola flavescentella, 211
Tineola furciferella, 211
Tomato parthenocarpy test, 268
Toxaphene, 247
Tranzschelia punctata, 125
Triatoma infestans, 359
Tribolium castaneum, 365
Tribolium confusum, 365
2,2,2-Tribromo-1,1-diphenylethane, 314
2,4,6-Tribromophenoxyacetic acid, 283

Trichlorobenzene, 390
2,3,6-Trichlorobenzoic acid, 269
2,2,2-Trichloro-1,1-bis-(4-bromo-2-methylphenyl)-ethane, 343, 344
2,2,2-Trichloro-1,1-bis-(4-bromo-3-methylphenyl)-ethane, 344
2,2,2-Trichloro-1,1-bis-(5-bromo-2-methylphenyl)-ethane, 343, 344
2,2,2-Trichloro-1,1-bis-(4-chloro-2-methylphenyl)-ethane, 343, 344
2,2,2-Trichloro-1,1-bis-(4-chloro-3-methylphenyl)-ethane, 344
2,2,2-Trichloro-1,1-bis-(5-chloro-2-methylphenyl)-ethane, 343, 344
1,2,2-Trichloro-1,1-bis-(4-chlorophenyl)-ethane, 327, 347
2,2,2-Trichloro-1,1-bis-(2-chlorophenyl)-ethane, 325
2,2,2-Trichloro-1,1-bis-(3-chlorophenyl)-ethane, 323
2,2,2-Trichloro-1,1-bis-(4-chlorophenyl)-ethane, 308
2,2,2-Trichloro-1,1-bis-(4-fluorophenyl)-ethane, 314, 347
2,2,2-Trichloro-1,1-bis-(2-hydroxy-3,5-dichlorophenyl)-ethane, 337
2,2,2-Trichloro-1,1-bis-(4-hydroxyphenyl)-ethane, 331
2,2,2-Trichloro-1,1-bis-(4-methoxy-3,5-dichlorophenyl)-ethane, 337
2,2,2-Trichloro-1,1-bis-(1-naphthyl)-ethane, 336
2,2,2-Trichloro-1,1-bis-(4-phenylphenyl)-ethane, 331
1,2,2-Trichloro-1,1-diphenylethane, 313
2,2,2-Trichloro-1,1-diphenylethane, 308
2,3,3-Trichloro-1,1-diphenylpropylene, 313
3,2,2-Trichloro-1,1-diphenylpropylene, 325
2,4,5-Trichlorophenoxyacetic acid, 184, 264, 281
2,4,6-Trichlorophenoxyacetic acid, 281
α-(2,4,5-Trichlorophenoxy)propionic acid, 288
Trichophaga abruptella, 211
Trichophaga percna, 211
Trichophaga tapetzella, 211
Trichoplusia ni, 363
Triethanolamine silicofluoride, 243
Trifolium pratense, 296
Trifolium repens, 296
Trihedralization of DDT analogues, 345
Triosephosphate dehydrogenase, 389
Triphenylmethane mothproofing agents 243
Trithion, 394
Trogoderma ornatum, 210
Trogoderma sternale, 210
Trogoderma versicolor, 210
Tropic acid, 274
Turnip maggot, 137
Twin fluid atomizers, 32, 90

U

Urea, 230
Uric acid, 230
Urtica urens, 295
Ustilago avenae, 111, 114
Ustilago crameri, 111

V

Vasates schlechtendali, 397
Venturia inaequalis, 119, 126
Venturia pyrina, 119, 120, 126
Vibrio comance, 118

W

Weeds, 297
Wetting agents, 57
Wheat bulb fly, 139
Wheat curl mite, 168
Wireworms, 138
Wool, chemical composition of, 219
  digestion of, 221
  structure of, 218
Wool-feeding insects, control of, 239

X

Xanthine oxidase, 229, 231
Xenopsylla cheopis, 393

Z

Zinc, uptake by spores, 123

Advances in Pest Control Research

CUMULATIVE INDEX

Volumes I–II

VOL. PAGE
Arthropods, Biting, Repellents for (Shambaugh, Brown, and Pratt) I 277
Bioassay of Pesticide Residues (Yun-Pei Sun) I 449
Chemical Analysis of Pesticide Residues (Schechter and Hornstein) I 353
Chemical Structure and Activity of DDT-Analogues with Special Consideration of Their Spatial Structures (Riemschneider) II 307
Chemical Structure, Relation to Activity for the 2,4-D Type Herbicide and Plant Growth Regulator (Wain) II 263
Chemistry and Action of Organic Phosphorus Insecticides (Fukuto) I 147
Chemistry and Mode of Action of Herbicides (Crafts) I 39
Control of Soil Fungi, Recent Advances in (Kendrick and Zentmyer) I 219
2,4-D Type Herbicide and Plant Growth Regulator, Relation of Chemical Structure to Activity for (Wain) II 263
DDT-Analogues, Chemical Structure and Activity of, with Special Consideration of Their Spatial Structures (Riemschneider) II 307
Fluid Kinetics of the Application of Pesticidal Chemicals (Fraser) II 1
Fungicides, Innate Toxicity of (McCallan and Miller) II 107
Fungitoxicity, Mechanisms of (Horsfall) I 193
Health Hazards, Associated with the Use of Pesticides, Control of (Barnes) I 1
Herbicide and Plant Growth Regulator, 2,4-D Type, Relation of Chemical Structure of Activity for (Wain) II 263
Herbicides, Chemistry and Mode of Action of (Crafts) I 39
Insecticide Resistance in Pest Species, Spread of (Brown) II 351
Insecticides, Organic Phosphorus, Chemistry and Action of (Fukuto) I 147
Insecticides, Seed and Soil Treatment with (Reynolds) II 135
Isotope Dilution Techniques for the Determination of Pesticide Residues (Redemann and Meikle) II 183
Mothproofing, Wool Digestion and (Waterhouse) II 207
Organic Phosphorus Insecticides, Chemistry and Action of (Fukuto) I 147
Pest Control Practices, Status of Systemic Insecticides in (Ripper) I 305
Pesticidal Chemicals, Fluid Kinetics of Application of (Fraser) II 1
Pesticide Research, Uses of Radioisotopes in (Dahm) I 81
Pesticide Residues, Bioassay of (Yun-Pei Sun) I 449
Pesticide Residues, Chemical Analysis of (Schechter and Hornstein) I 353

	VOL.	PAGE
Pesticide Residues, Isotope Dilution Techniques for the Determination of (Redemann and Meikle)	II	183
Pesticides, Control of Health Hazards Associated with the Use of (Barnes)	I	1
Pest Species, Spread of Insecticide Resistance in (Brown)	II	351
Radioisotopes, Uses of, in Pesticide Research (Dahm)	I	81
Repellents for Biting Arthropods (Shambaugh, Brown, and Pratt)	I	277
Seed and Soil Treatment with Insecticides (Reynolds)	II	135
Soil Fungi, Control of, Recent Advances in (Kendrick and Zentmyer)	I	219
Systemic Insecticides, Seed and Soil Treatment with (Reynolds)	II	135
Systemic Insecticides Status of in Pest Control Practices (Ripper)	I	305
Toxicity, Innate, of Fungicides (McCallan and Miller)	II	107
Wood Digestion and Mothproofing (Waterhouse)	II	207

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